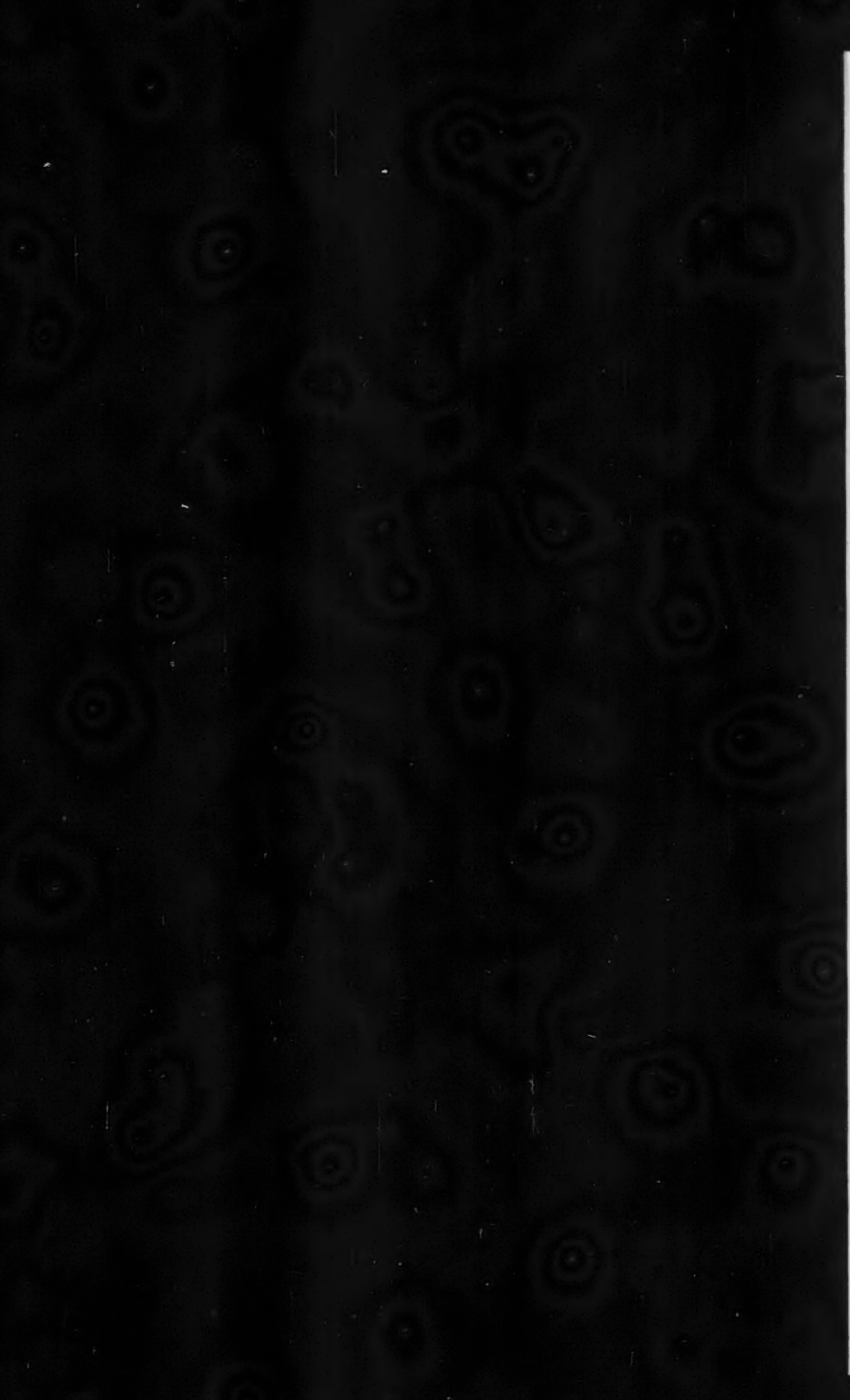
CANADIAN JOURNAL OF PLANT SCIENCE

VOLUME 39

January, April, July and October
1959

EDITED BY THE
AGRICULTURAL INSTITUTE OF CANADA
OTTAWA, CANADA

Published quarterly by the authority of the
Chairman of the National Committee on Agricultural Services



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CANADIAN JOURNAL OF PLANT SCIENCE

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Authorized as second-class mail, Post Office Department, Ottawa

CANADIAN JOURNAL
OF PLANT SCIENCE

VOLUME 39 JANUARY 1959 No. 1

INHERITANCE OF BLUE KERNEL COLOUR IN WHEAT[1]

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[Received for publication July 25, 1958]

ABSTRACT

The inheritance of blue kernel colour was studied in crosses of a blue-kernelled *Agropyron elongatum* (Host) Beauv. X *Triticum vulgare* (Vill.) Host. derivative, Blue 1, with Rescue, a red-kernelled variety, and with Lemhi a white-kernelled variety. The blue colour was found to be in the endosperm and xenia was observed. Each generation from F_1 to F_3 was grown at a different location under varying environments. From the ratios obtained it was concluded that blue colour is conditioned by two complementary dominant genes whose expression appears to be influenced by environment.

INTRODUCTION

In the search for new germ plasm for wheat-breeding projects, species of *Agropyron* are frequently used. Since *Agropyron* genes should be of value in this work, a study of their mode of inheritance in crosses with *Triticum vulgare* (Vill.) Host. is important. The blue kernel colour of *Agropyron* has been transferred to wheat by backcrossing. The present study was conducted to determine the mode of inheritance of blue kernel colour.

The inheritance of red kernel colour of the seed coat in wheat has been reported by many workers since the time of Biffen (1) in 1905. Kattermann (6) described a *vulgare* type wheat, probably wheat-rye in origin, with blue-green or blue-grey kernels in which the colour was the result of anthoxanthin in the endosperm. In crosses between this strain and a white kernelled variety, where the blue was used as the maternal parent, the immediate effect of pollen (xenia) on endosperm colour was observed. Kernels from this cross were white while those from the self-pollinated maternal parent were blue. In the reciprocal cross, no influence of pollen was noted. From observations made on the seed from the immediate cross (F_1 seed) and the seed from the F_1 plants (F_2 seed), Kattermann concluded that there were two pairs of factors, namely a colour factor F for blue kernel in the blue strain and an inhibiting factor H in the white variety. Endosperm characters in grass seeds are determined by a triploid genetic make-up. In the process of double fertilization one sperm nucleus unites with the egg nucleus to initiate the embryo, while the other sperm

[1] Contribution No. 213, Cereal Crops Division, Experimental Farms Service, Ottawa; from a thesis submitted to the University of Saskatchewan in partial fulfilment of the requirements for the Degree of Master of Science.

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nucleus fuses with the two polar nuclei to form the primary endosperm nucleus. Kattermann's endospermic characters are expressed as fractions, ffHH/fH and FFhh/Fh, where the numerator represents the female gamete and the denominator represents the male. The only types that Kattermann found to be stable for blue were considered to have the genotype FFhh/Fh and this combination appears only once in 16 times. A second group, which gave some blue kernels, Kattermann classified as labile or unstable. These were presumed to be of the genotypes FFhh/fh, ffhh/Fh and FFhh/fH. The remainder were white or of a much lighter blue.

Xenia is common in corn where many genes are known to be associated with aleurone colour. Some of these are basic to the formation of purple and red pigment, according to Emerson *et al.* (3, 5).

Many pigments have been identified in plants. Of these anthocyanin and anthoxanthin are important in determining darkness of colour. Anthocyanin, by far the most common, is frequently concentrated in the portion of the plant most exposed to light (9). In some cases, it acts as an indicator of the pH of the cell sap. The explanation of the labile nature of these pigments, as reported by Kattermann, was given by Scott-Moncrieff (9) in 1924 and more recently by Gortner and Gortner (4). They showed anthocyanins to be a complex system of ring structures in which one of the carbon bonds may have a sugar or a hydroxyl group. The presence of either the sugar or hydroxyl group makes the colour bluer. For example, the addition of either group to a molecule would change scarlet to maroon, and the addition of both would give purple.

MATERIALS AND METHODS

According to Suneson and Pope (11), Sando made the cross *Agropyron elongatum* x *Triticum vulgare* from which the material for the present study was derived. Suneson grew this material for 10 years, during which time he crossed and backcrossed it to the common wheat variety Baart. In 1949 some of the blue kernel material was introduced to the Experimental Farm, Lethbridge. Cytological examination of Blue 1, the plant used in this study, showed it to have 42 chromosomes, composed of 19 pairs and a ring of 4*.

Reciprocal crosses between Blue 1 and Lemhi, and Blue 1 and Rescue, were made during the summer of 1949 in the irrigated nursery at Lethbridge. Plants from these seeds were grown in pots in the greenhouse at the University of Saskatchewan the following winter. Daylight was supplemented by artificial light.

Of the material harvested in the greenhouse, 10 dark-blue, 10 light-blue and 10 non-blue seeds from each F_1 plant were space seeded in the field at Regina. A large number of spikes were bagged to ensure selfing. Because of fall frost, classification of seed colour of some of these F_2 plants was difficult and seed of these was discarded.

Fisher's chi-square analysis was used in testing the goodness of fit of the data to the hypothesized ratios.

* Larson, R. I. *Personal communication.*

RESULTS

The three generations studied were grown under widely different conditions. The crosses were made on plants grown under irrigation at Lethbridge. The F_1 plants were grown in the greenhouse at Saskatoon with high moisture and humidity, while the F_2 were grown under field conditions at Regina. The differing environmental conditions appeared to influence the proportion of blue seed obtained. Available moisture in the irrigated nursery was intermediate between the high moisture of the greenhouse and the low moisture of the field at Regina. Since the greenhouse crop was grown in the fall and early winter, light intensity was low as compared with the field conditions at Lethbridge and Regina.

Blue 1—Lemhi Cross

All seeds resulting from the cross Blue 1 x Lemhi were blue. However, in the reciprocal cross, 3 seeds were blue and 25 were non-blue, indicating a partial effect of xenia. In the F_2 a good fit was obtained to a 9:7 ratio, the ratio not being influenced by the direction of the cross (P values 0.10-0.20 in both cases). F_1 and F_2 data are presented in Table 1.

The results obtained for plants grown from blue F_2 kernels are given in Table 2. A total of 122 plants were classified for seed colour. Of these, 60 appeared to segregate in a ratio of 1 blue : 1 non-blue; 49 in a ratio of 1 blue : 3 non-blue; and 13 bred true for blue. In the group segregating in a 1:1 ratio, a total of 1571 kernels were blue and 1503 were non-blue. This is a satisfactory fit to the 1:1 ratio (P value 0.20-0.30). The other segregating group, with 768 blue and 2425 non-blue, gave a good fit to the 1:3 ratio (P value 0.20-0.30).

Blue 1—Rescue Cross

In the reciprocal crosses between Rescue and Blue 1, all seed was blue regardless of the direction of the cross. The F_2 population segregated 577 blue to 934 non-blue. The F_1 and F_2 data are shown in Table 1.

The results obtained when the progeny of blue F_2 seeds were grown are presented in Table 2. Of the 44 plants classified, 20 appeared to segregate in a 1 blue : 1 non-blue ratio for kernel colour, and 19 in a 1 blue : 3 non-blue ratio. The remaining 5 bred true for blue. In the first class, 583 seeds were blue and 589 non-blue, a good fit to the 1:1 ratio (P value 0.85-0.90). In the second class, 257 seeds were blue and 657 non-blue.

TABLE 1.—FREQUENCIES OF BLUE AND NON-BLUE KERNELS IN THE F_1 AND F_2 OF CROSSES OF BLUE 1 WITH LEMHI AND RESCUE

Cross	F_1		F_2	
	Blue	Non-blue	Blue	Non-blue
Blue 1 x Lemhi	40	0	470	407
Lemhi x Blue 1	3	25	579	496
Blue 1 x Rescue	27	0	336	514
Rescue x Blue 1	46	0	241	420

TABLE 2.—CLASSES OF F_2 PLANTS ACCORDING TO THEIR F_3 KERNEL SEGREGATIONS

Cross		No. of plants breeding 1 blue: 1 non-blue	No. of plants breeding 1 blue: 3 non-blue	No. of plants breeding All blue	P
Blue 1-Lemhi	Actual	60[1]	49[2]	13	
	Expected (4:4:1)	54.22	54.22	13.56	0.50–0.70
Blue 1-Rescue	Actual	20[3]	19[4]	5	
	Expected (4:4:1)	19.56	19.56	4.88	0.95–0.99

Ratios for kernel colour within segregating classes

[1]L x Bl =	1033 : 931	
Bl x L =	538 : 572	
	1571 : 1503	P for 1:1 ratio = 0.20-0.30
[2]L x Bl =	639 : 1894	
Bl x L =	129 : 531	
	768 : 2425	P for 1:3 ratio = 0.20-0.30
[3]R x Bl =	146 : 172	
Bl x R =	437 : 417	
	583 : 589	P for 1:1 ratio = 0.85-0.90
[4]R x Bl =	74 : 206	
Bl x R =	183 : 451	
	257 : 657	P for 1:3 ratio = 0.03

This approaches the 1:3 ratio more closely than any other common genetic ratio (P value 0.03). Forty non-blue F_2 seeds were sown and the resulting plants bagged to ensure selfing. Of these, 11 segregated 1 blue : 3 non-blue, while 29 bred true for non-blue.

When seeds of different intensities of the blue were sown, that is dark blue, intermediate blue and light blue, it was observed that segregation occurred in the progeny of all three types in approximately the same proportions. Neither the direction of the cross nor the parent involved appeared to influence the segregations. This is shown in Table 3 by the similarity of the ratios of blue : non-blue. A few mottled seeds were found in the study. Since their progeny segregated in a similar manner to those of the blue kernel they were placed in this group.

DISCUSSION AND CONCLUSIONS

Data from the crosses indicate that there are two dominant complementary genes for blue colour in Blue 1. Two complications enter into this study. First, ratios are determined by a double allotment of genes from the maternal parent and the effect of xenia on the endosperm cells. Second, the labile nature of blue colour tends to mask the mode of inheritance. F_1 seed of the same genotype, derived from reciprocal crosses, may have different endosperm colour. The difference in the composition of the embryo and the endosperm can be illustrated as follows:

(a) AABB x aabb – – – AaBb (embryo) – – – AABB/ab (endosperm)

(b) aabb x AABB – – – AaBb (embryo) – – – aabb/AB (endosperm).

It was found that, under all growing conditions, an endosperm with four dominants, as in (a), was blue, while one with only two dominants, as in (b), could be either blue or non-blue depending on the environment. The

TABLE 3.—RATIOS OBTAINED IN COLOUR INTENSITY PROGENY TESTS (F_2 PLANTS)

Cross	Dark blue		Intermediate blue		Light blue		Av. of blue	
	B	Non-B	B	Non-B	B	Non-B	B	Non-B
Lemhi x Blue 1	1	1.5	1	1.5	1	1.7	1	1.5
Blue 1 x Lemhi	1	1.4	1	1.3	1	1.0	1	1.2
Rescue x Blue 1	1	0.9	1	0.9	1	1.3	1	1.1
Blue 1 x Rescue	1	1.0	1	1.0	1	1.0	1	1.0

TABLE 4.—F_2 GENOTYPES AND THE EXPECTED RATIO OF THE CORRESPONDING F_3 ENDOSPERMS

F_2 embryo	Possible combinations derived from:		Possible F_3 endosperm genotypes[1]	Ratio when 2 of each dominant gives blue
	Polar nuclei	Pollen		
1 AABB	AABB	AB	AABB/AB	All blue
2 AABb	AABB	AB	AABB/AB,AAbb/AB	
	AAbb	Ab	AABB/Ab,AAbb/Ab	1 : 1
2 AaBB	AABB	AB	AABB/AB,aaBB/AB	
	aaBB		AABB/aB,aaBB/aB	1 : 1
4 AaBb	AABB	AB	AABB/AB,AAbb/AB, aaBB/AB,aabb/AB	
	AAbb	Ab	AABB/Ab,AAbb/Ab, aaBB/Ab,aabb/Ab	
	aaBB	aB	AABB/aB,AAbb/aB, aaBB/aB,aabb/aB	
	aabb	ab	AABB/ab,AAbb/ab, aaBB/ab,aabb/ab	1 : 3

[1] The underlined genotypes are of blue phenotype.

wide variety of conditions under which the three generations were grown, though complicating this study, made it possible to demonstrate the labile nature of the blue pigment.

Blue 1—Lemhi Cross

The Blue 1 x Lemhi cross produced 40 blue F_1 kernels whose endosperm genotype may be represented by AABB/ab, while the genotype of the reciprocal cross, which produced 3 blue and 25 non-blue kernels, would be represented as aabb/AB. The fact that 3 kernels of the genotype aabb/AB were blue indicates that, under the environmental conditions at Lethbridge, blue colour could occasionally be expressed when only one of each of the complementary dominant genes was present. This same gene dosage under greenhouse conditions gave complete expression of the blue colour as shown by the 9:7 ratio of blue to non-blue kernels in the F_2.

TABLE 5.—EXPECTED ENDOSPERM GENOTYPES[1] FROM A PLANT OF THE CONSTITUTION AaBb (F_2 SEGREGATION FROM RESCUE-BLUE 1 CROSS)

		Male genotypes in equal proportions			
		AB	Ab	aB	ab
Female genotypes in equal proportions	AABB	AABB/AB	AABB/Ab	AABB/aB	AABB/ab
	AAbb	AAbb/AB	AAbb/Ab	AAbb/aB	AAbb/ab
	aaBB	aaBB/AB	aaBB/Ab	aaBB/aB	aaBB/ab
	aabb	aabb/AB	aabb/Ab	aabb/aB	aabb/ab

[1] The underlined genotypes are of blue phenotype when 4 dominants are required.

Since two segregating groups, 1:1 and 1:3, were observed in the F_3 seed it is concluded that at least two of each dominant are required to express the blue colour under the environment found in the field at Regina. In Table 4 it is shown that when Blue F_2 seeds are sown the resulting plants segregate for seed colour in a ratio of four plants segregating 1:1 to four segregating 1:3 to one breeding true for blue kernel colour. The actual and expected ratios of seed colour and the actual and expected numbers of plants in each segregating class are presented in Table 2. The numbers of plants in each group fit the expected 4:4:1 ratio with a P value of 0.50—0.70.

Blue 1—Rescue Cross

In the Blue 1–Rescue crosses, only blue seed was produced. Under the environment of the irrigated nursery at Lethbridge only one of each dominant factor in the endosperm was required to express blue colour. However, in the F_2 segregation the data do not fit the 9:7 ratio but fit a 6:10 ratio with a P value of 0.06 (Table 5). This is the ratio which would be expected when the presence of any four dominants in the endosperm conditions blue colour.

Some of the non-blue F_2 seeds would have embryos with both dominants. Since two of each dominant seemed to express blue colour in F_3, then some non-blue F_2 plants should segregate in F_3. Actually 11 segregated 1 blue : 3 non-blue, and 29 bred true for non-blue when selfed. The expected ratio is 3 segregating : 7 non-segregating (see Table 6). The observed segregation closely fits the 3:7 ratio (P value 0.70—0.80).

The F_3 from the blue F_2 seeds segregated in much the same manner as the F_3 from blue F_2 seeds in the Blue 1—Lemhi crosses. Approximately equal numbers of plants segregated 1:1 as segregated 1:3. Another smaller group bred true for blue. The actual and expected numbers of plants in each segregating group are presented in Table 2. The data fit the expected 4:4:1 ratio with a P value of 0.95-0.99.

Environmental Influence

Blue colour appears to be controlled by complementary pairs of factors whose expression can be modified by environmental conditions. For example, the genotype represented by aabb/AB in the Lemhi x Blue 1 endosperm was mostly white at Lethbridge, blue in the greenhouse at

TABLE 6.—ENDOSPERM AND EMBRYO GENOTYPES, ENDOSPERM PHENOTYPES AND EXPECTED SEGREGATION IN NEXT GENERATION

Endosperm genotype	Embryo genotype	Endosperm phenotype	Segregation on selfing (blue: non-blue)
AABB/AB	AABB	Blue	All blue
AABB/Ab	AABb	Blue	1 : 1
AABB/aB	AaBB	Blue	1 : 1
AABB/ab	AaBb	Blue	1 : 3
AAbb/AB	AABb	Blue	1 : 1
AAbb/Ab	AAbb	Non-blue	Non-blue
AAbb/aB	AaBb	Non-blue[1]	1 : 3
AAbb/ab	Aabb	Non-blue	Non-blue
aaBB/AB	AaBB	Blue	1 : 1
aaBB/Ab	AaBb	Non-blue[1]	1 : 3
aaBB/aB	aaBB	Non-blue	Non-blue
aaBB/ab	aaBb	Non-blue	Non-blue
aabb/AB	AaBb	Non-blue[1]	1 : 3
aabb/Ab	Aabb	Non-blue	Non-blue
aabb/aB	aaBb	Non-blue	Non-blue
aabb/ab	aabb	Non-blue	Non-blue

[1] Segregating non-blue types.

Saskatoon, and white in the field in Regina. In the greenhouse, moisture conditions were almost ideal but the intensity of light was low. At Lethbridge moisture conditions were less favourable. Sprinkler irrigation supplied additional moisture but hot winds and dry air made humidity low and transpiration high. At Regina in 1950 the field conditions were drier than the irrigated nursery conditions the year before at Lethbridge.

Previous studies have shown that, when adequate water is supplied to plants, the expression of blue colour is more intense (4, 9). Gortner and Gortner state that almost invariably pigments of red, violet and blue colours belong to the anthocyanin group (4). Violet and blue colour in barley has been established as due to anthocyanin (10). Since the blue aleurone in this study is probably caused by anthocyanin, a more acid condition would decrease the colour intensity. According to Newton and Martin (7) plants become more acid with lack of moisture. They also claim that an increase in light will increase pigmentation. Robinson (8) states that low temperature, high intensity of light and excess of oxygen favour the appearance of pigments.

It is interesting to note the apparent differential response to light and moisture between the two crosses, Blue 1 x Lemhi, and Blue 1 x Rescue. In the Lemhi cross, abundant moisture appeared to be the most important factor for the expression of blue colour. Full expression occurred under the high moisture conditions of the greenhouse in plants having two or more complementary dominant genes, while in the irrigated nursery blue colour was only occasionally expressed with two complementary dominants. In the Rescue cross, on the other hand, the expression of blue colour appeared to be more dependent on light intensity. Under the irrigated nursery conditions, only two complementary dominants were required for complete expression, whereas under much reduced light in the greenhouse four dominants were necessary.

No information is available on other environmental influences such as soil fertility. Bonner states that it is known that a deficiency of nitrogen or phosphorus causes an increase in anthocyanin but the manner in which this is brought about is not understood (2). It is unlikely, however, that spaced plants grown on well prepared summerfallow or in potting soil would be deficient in either of these elements.

The answer as to why certain of these genotypes show a different phenotype under varying environmental conditions may be attributed to a combination of light and moisture. The variability of these factors seems to explain the inconsistency in expression of blue colour.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to B. C. Jenkins, who introduced the Blue 1 line and made the original crosses used in this study, and to L. H. Shebeski for his advice. Indebtedness is expressed to the author's wife, Vera, who assisted with the field work.

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THE CONTROL OF GROWTH AND DEVELOPMENT IN RED CLOVER (*TRIFOLIUM PRATENSE* L.)

I. RECORDING AND ANALYSIS OF DEVELOPMENTAL PATTERNS IN MORPHOGENESIS[1,2]

BRUCE G. CUMMING[3]

[Received for publication November 21, 1957]

ABSTRACT

A key is described that provides a simple method for recording the sequential distribution of all leaves, buds, and inflorescences visible on a primary stem. Numbers are assigned to nodes in a basipetal sequence with each flowering node as number 1.

In the "first cycle of growth" of a primary bud the number of nodes on each lateral flowering shoot was found to be positively correlated with, and linearly related to, the number of the node at which each was borne. It was also found that both the number of buds, non-flowering and flowering shoots, [T], and the number of inflorescences, [I], were linearly related to primary node number. Indoleacetic acid (IAA), 2,3,5-triiodobenzoic acid (TIBA), and maleic hydrazide amine (MH), altered the amount of lateral bud and shoot growth but not the linear relationships.

Attainment of "ripeness-to-flower" coincident with production of a systemic non-polar florigenic stimulus could result in the observed conformity to theoretically "expected node numbers for flowering" on all lateral shoots.

Treatment with 800 p.p.m. MH caused abortion of inflorescences and hastened onset of the "second cycle of vegetative growth" through cessation of growth and differentiation of "first cycle" floral apices; virus infection has caused similar effects.

INTRODUCTION

It is being increasingly recognized that significant advances in the control of plant growth under the various agricultural systems depend, to a considerable extent, upon a critical understanding of the changes which can occur in the rate and pattern of development of the plant.

This implies a need for basic knowledge of the potentialities for growth and development inherent in a particular genotype. Conversely, a critical understanding is required of the dimensions of the inter-relationships between individual factors of the external environment and the significant features of a plant's morphogenesis.

During investigations extending over the period from 1952-1956 (5), the author studied some of the influences of light intensity, photoperiod, temperature, and growth regulators, on growth and developmental patterns in red clover. Certain aspects of morphogenesis have been related to the influence of a primary endogenous factor: auxin. Arising from these studies it has been possible to obtain improved methods in vegetative propagation and in the control of growth and development.

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[1] Based on part of a Ph.D. thesis accepted by the Faculty of Graduate Studies and Research, McGill University, Montreal, Que., May, 1956.

[2] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Journal Series No. 437.

[3] Associate Botanist, Botany and Plant Pathology Laboratory, Science Service, Canada Department of Agriculture, Ottawa, Ont.

REVIEW OF LITERATURE

In recent years several workers have undertaken detailed physiological and morphogenetic analysis of developmental patterns shown by crop plants. The most critical studies have been undertaken with species of the Gramineae (e.g. 3,4,8,17,18,22,23).

Lang (16) has emphasized that, of the various stages in flowering, floral initiation is by far the most fundamental, since it marks the actual transition from vegetative to reproductive development. Klebs (14) referred to apices capable of such a transition as being "ripe-to-flower".

Red clover is classified as a long-day plant (LDP). Plants normally fail to flower under short daylengths (SD), i.e. under photoperiods below a certain critical value [non-photoinductive cycles (9)]. This value depends on the variety, type, population, or clone involved (13,19,27). Two reports (1,11) have given evidence that suggests the transmission of a non-polar flowering stimulus in red clover. Transfer of such a stimulus from donor branches under SD was shown by the formation of inflorescences on receptor branches (1). *Orobanche minor*, a total parasite of red clover, was found to flower only when the host—red clover plants—did so (11).

Leaf number can be employed as a criterion of the relative amount of vegetative growth preceding the first flower (22,23). Golubev (7) recommended using the number of internodes on red clover stems as a basis for classification of plants. Hawkins (10) has observed that differences in number of internodes on the main stem enable the different types ("con-varieties") of red clover to be distinguished with precision. As compared to recording actual times of flowering, this method has the advantages of being more critical and more independent of seasonal conditions; also, data can be recorded within wider time limits. Mitchell (20), in studies on ryegrass, has used diagrammatic representations to record differences in leaf and tiller formation. He has termed successive leaves upon the main stem: leaf 1 (L1), L2, etc. L1 is the first leaf on the main stem above the coleoptile, then L2, and so on. Tillers from the axillary bud of each leaf are similarly numbered, i.e. T1 at L1, T2 at L2, etc. Leaves on Tiller 1 are referred to as T1L1, T1L2 etc. An axillary bud on T1L2 developing to a tiller is called T1T2.

The number of internodes and lateral shoots may vary in red clover according to variety and seasonal conditions (15,28). Bird (2) recognized five discrete growth types occurring within the early-flowering class of red clover grown in Quebec. The basis for classification was rosette (crown) development, stem elongation and flowering of seedlings. Seedlings were transplanted to the field in early summer and classified in October of the same year. The five growth types were more conveniently described by Steppler and Raymond (24) as:

Type O—produces rosette early, in year of establishment; no flower formation.

Type I—produces strong rosette in the year of establishment with one or a very few prostrate flower stems.

Type II—produces fairly prominent rosette in the year of establishment with a ring of flower stems, generally prostrate.

Type III—produces indistinct rosette in year of establishment with many flower stems, generally upright.

Type IV—produces no rosette in year of establishment; many sparsely-leaved upright flower stems.

Auxins are known to inhibit the growth of lateral buds on plants in the phenomenon of apical dominance (26). In red clover (5) variations in the expression of apical dominance have been shown to result from differences in endogenous auxin level. These differences were brought about experimentally either by changing the daylength to which plants were exposed or by application of auxin (IAA) or anti-auxin (TIBA) solutions.

MATERIALS AND METHODS

Unless otherwise stated, all plants were grown under natural daylength at Macdonald College (latitude *ca.* 45° 40′ N). To decrease variability between plants all studies were conducted on non-vernalized "propagules"* of clonal material derived from selected genotypes of the Dollard variety of medium-early red clover. The selected genotypes were classified according to growth type (24).

The following growth regulators were used for spraying spaced plants under field conditions: 3-indoleacetic acid (IAA), maleic hydrazide amine (MH), 1-naphthaleneacetic acid (NAA), 2,3,5-triiodobenzoic acid (TIBA).

Control and growth regulator sprays included a wetter, ultrawet 60L (in concentrated liquid form).** Sprays were applied with a 3-gallon capacity air-compression sprayer and were as follows:

50 IAA	=	once-weekly spray with 50 p.p.m. IAA from June 22 to July 28, 1955.
MH 800	=	once-weekly spray with 100 p.p.m. MH from June 22 to July 6; 300 p.p.m. MH from July 13 to July 28; 800 p.p.m. MH on Aug. 6 and Aug. 23, 1955.
50 NAA	=	once-weekly spray with 50 p.p.m. NAA from June 22 to July 28, 1955.
NAA 50	=	sprayed with 50 p.p.m. NAA on Aug. 6 and Aug. 23, 1955.
TIBA 25	=	sprayed with 25 p.p.m. TIBA on Aug. 6 and Aug. 23, 1955.
TIBA 100	=	sprayed with 100 p.p.m. TIBA on Aug. 6 and Aug. 23, 1955.

RESULTS

Key for Recording Morphogenesis

The units for growth and development in red clover are the primary buds that comprise the "rosette" arising from the crown of the tap root.

* "Propagule" is defined for this context as: any unit for vegetative propagation capable of producing a normal mature plant.

** Naugatuck Chemicals Ltd., Elmira, Ont.

For any particular genotype an understanding of yield potentiality can therefore be obtained, in part, from a critical analysis of morphogenesis of a primary bud under any given range of conditions. When isolated as a propagule, any bud of red clover which is in the non-determinate vegetative stage of growth may fulfil the role of a seedling—once it has acquired independence through production of a root system. A "cycle of growth" is defined here as "the phases of growth and development from the origin of a bud to the stage including its development to produce mature flowers (with or without seed)". A "cycle of vegetative growth" is defined as "the cycle of growth that includes all phases from the origin of a bud, excluding initiation and formation of floral parts".

With the exception of the apical flowering node, only one leaf arises directly from each node upon a stem of red clover. Each succeeding leaf upon a stem arises alternately but in the same plane as the previous one. On a flowering stem, generally two, but sometimes only one, sessile or short petioled bract-like leaves arise from the apical node subtending the terminal inflorescence head—which is sessile or short-peduncled. This apical node will be referred to as the "flowering node". The number of leaves upon a flowering stem is, therefore, equal to the number of nodes (n)-1, if any leaves at the flowering nodes are excluded, but either n or n+1 if the latter are included.

The numbers, measurements, and location of all major visible organs upon a stem can be recorded by use of the key described below.

The key for an entire primary stem is shown in Figure 1, together with its diagrammatic representation. This represents the buds, stems, and inflorescences that had arisen from a primary "crown" bud of a field planted propagule (planted June 10, 1955, data recorded Aug. 9, 1955). Node numbers are assigned in a basipetal direction with the apical node as 1, the basal node n. In the method of Mitchell (20) node numbers were assigned in an acropetal direction.

Upper case letters are used in the key to represent the different stages of development of buds and shoots:

B = bud.

E = elongating shoot without visible terminal inflorescence (i.e. non-flowering shoot).

G = shoot with green terminal inflorescence head (i.e. florets not receptive for pollination).

P = shoot with pink terminal inflorescence head (i.e. florets receptive for pollination).

M = shoot with mature terminal inflorescence head (i.e. florets brown, at stage of "seed-setting").

Ab = shoot with aborted terminal inflorescence head (i.e. non-functional).

The "index number" assigned to each upper-case letter represents the node number of the lower order shoot from which the particular lateral bud or shoot arose. The number immediately following each "index number" represents the number of nodes on each respective flowering shoot.

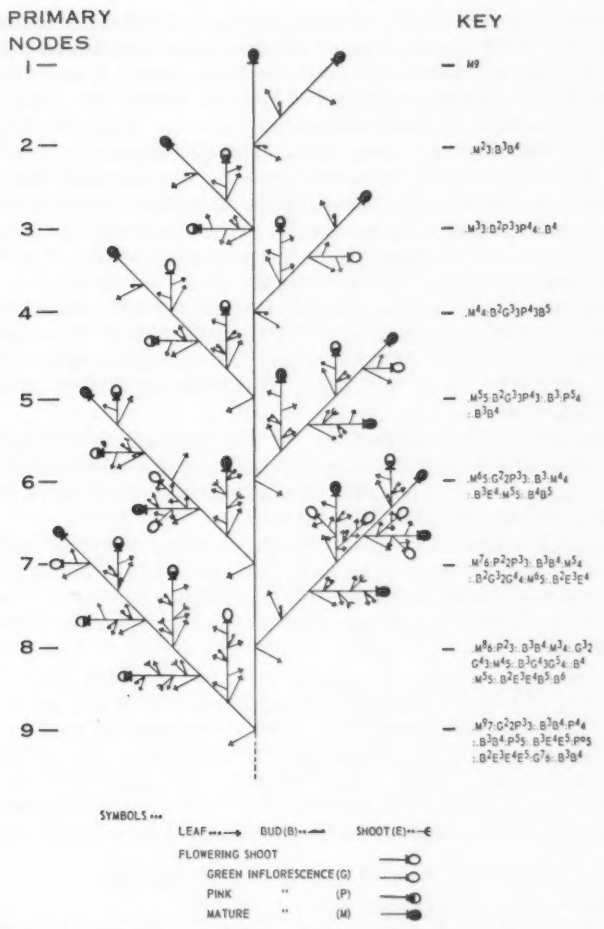

FIGURE 1. Key and diagrammatic representation of entire primary stem. Recorded August 9, 1955 from field planted propagule. Clone 9.

The number of nodes on buds and non-flowering shoots cannot be given accurately without microdissection. However, the number of leaves can be used as an estimate of number of nodes, but this may be less than the value finally realized.

Referring to Figure 1, M²3 represents a shoot with a mature terminal inflorescence head, having three nodes, that is borne at the second primary node. In this notation, the value for number of nodes on a lateral shoot does not include the point of origin of the lateral on the shoot. However, when laterals of the next higher order arise from that point they receive a supplementary index number to indicate their position. Thus, in the

above instances, .$M^{2}3:B^{3}B^{4}$ indicates that one second-order lateral bud was borne at the third (basal) node of the first-order lateral shoot and one at the point of origin of this lateral on the primary stem. A single dot precedes all first and succeeding order lateral buds or shoots, two dots precede all second and succeeding order laterals, and so on. With this notation it is understood that dots of a given order extend to the next dots of whatever order. When the succeeding dots are of a higher order, this shows inclusion of a lateral on the respective stem indicated by the preceding dots.

In actual recording, the key can be written as a single group or formula in the sequence as in Figure 1, from the primary flowering or non-flowering apex, downwards. Thus, the formula for the entire stem is:
$M9.M^{2}3:B^{3}B^{4}.M^{3}3:B^{2}P^{3}3P^{4}4:.B^{4}.M^{4}4:B^{2}G^{3}3P^{4}3B^{5}.M^{5}5:B^{2}G^{3}3P^{4}3:.B^{3}:P^{5}4:.$
$B^{3}B^{4}.M^{6}5:G^{2}2P^{3}3:.B^{3}:M^{4}4:.B^{3}E^{4}:M^{5}5:.B^{4}B^{5}.M^{7}6:P^{2}2P^{3}3:.B^{3}B^{4}:M^{5}4:.$
$B^{2}G^{3}2G^{4}4:M^{6}5:.B^{2}E^{3}E^{4}.M^{8}6:P^{2}3:.B^{3}B^{4}:M^{3}4:.G^{3}2G^{4}3:M^{4}5:.B^{3}G^{4}3G^{5}4::$
$B^{4}:M^{5}5:.B^{2}E^{3}E^{4}B^{5}:B^{6}.M^{9}7:G^{2}2P^{3}3:.B^{3}B^{4}:P^{4}4:.B^{3}B^{4}:P^{5}5:.B^{3}E^{4}E^{5}:P^{6}5:.$
$B^{2}E^{3}E^{4}E^{5}:G^{7}6:.B^{3}B^{4}.$

Summation of all numbers except the "indices" in this formula provides a value of 159—which, excluding bud and non-flowering shoot nodes, is the total number of nodes on the stems. The same value is obtained by a count taken from the diagram.

Although measurements of leaves, petioles, stems or inflorescences are not shown in the key, these were recorded when required by inserting values in a position appropriate to the particular symbols. Comparative sizes of the different organs and stems are not accurately represented in the diagram.

Use of the key made it possible to record in detail the sequences of developmental patterns produced in morphogenesis under varied environmental conditions and treatments. This eliminates the need for diagrams while still portraying the sequential distribution of leaves, shoots and inflorescences upon a stem. The method was found to be of particular value in recording data from stems with a large amount of lateral bud and shoot formation. In such cases diagrams would be difficult to construct.

Developmental Patterns Arising from the Primary Buds

(a) *Theoretical Expectations*

Some means other than microdissection were sought to define the determinacy conferred by floral initiation on growth and development of any one entire primary stem of red clover plants. Theoretically, a definite pattern of growth and development would be expected if it is assumed that each apex in vegetative growth is capable of producing a primary and axillary bud and that each in turn can repeat the sequence. Assuming the same rate of production and growth of succeeding order laterals, then, whatever the stage shown, the number of nodes on each lateral would correspond exactly with the node number at which it is borne.

If, then, a systemic non-polar influence resulted in floral initiation at more or less the same instance at all apices which have reached a certain stage in development ("ripeness-to-flower"), a definitive pattern for inflorescence production would be laid down. The features of this pattern are noted under (1), (2) and (3) in following text.

(b) *Analysis of Data for Primary Stems*

(1). Theoretically, in the regression formula:

$y^n = a^n + b^n x^n \ldots$ y^n = numbers of nodes on each flowering shoot.
x^n = node number at which each respective flowering shoot is borne.

The linear regression coefficient b^n would be equal to 1. The intercept a^n would be equal to zero ($a=y$ where $x=0$); i.e., a lateral shoot with one node can be produced at the first node. The paired values of y^n and x^n would be completely correlated. Thus, a lateral flowering shoot arising for example from the sixth node of a flowering stem would possess an inflorescence terminally upon a stem with six nodes, whatever the respective order of shoots involved. In the following discussion, this definitive sequence will be referred to as the "expected node numbers for flowering".

Determinacy to the maximum amount of lateral shoot growth would, therefore, be a function of the number of nodes on the primary stem at the time of floral initiation. For example, on a primary flowering stem with nine nodes, the maximum lateral shoot node number would be 9 and this lateral shoot would arise at primary node 9, i.e. the basal node on the primary stem.

Analysis of data recorded from entire primary stems of clones of different growth types, grown in the field under photoinductive cycles, are shown in Table 1, a and b. The data for

TABLE 1.—LINEAR REGRESSIONS: b^n – NUMBER OF NODES ON FLOWERING SHOOTS ON NODE NUMBER AT WHICH BORNE; $b[^I]$ – TOTAL NUMBER OF INFLORESCENCES AT EACH SUCCESSIVE PRIMARY NODE, [I], ON PRIMARY NODE NUMBER AT WHICH BORNE; $b[^T]$ – TOTAL NUMBER OF BUDS, NON-FLOWERING AND FLOWERING SHOOTS AT EACH SUCCESSIVE PRIMARY NODE, [T], ON PRIMARY NODE NUMBER AT WHICH BORNE

a. Observed data
b. Calculated data

a.

Date of field observation	Clone	Growth type	Number of nodes on primary stem	[I]	Total infls.	[T]	Total buds, shoots, infls.
1/9/55	469	IV	8	2,0,2,2,4,5,7,4	26	2,0,2,2,4,5,7,6	28
1/9/55	23	IV	8	1,1,2,3,4,5,3,10	29	1,1,2,4,4,5,4,11	32
13/9/55	26	III	7	1,1,2,3,5,5,6	23	1,3,4,6,12,8,14	48
27/8/55	339	III	8	1,2,4,5,8,11,11,10	52	1,4,4,6,11,15,18,15	74
1/9/55	12	II-III	9	1,1,3,4,3,8,8,12,18	58	1,1,3,4,8,9,11,16,20	73
29/8/55	9	I	9	1,1,3,3,4,5,7,9,7	40	1,3,5,5,8,8,13,18,20	81
12/9/55	64	O	9	1,1,3,4,8,4,7,4	32	1,3,3,4,9,5,12,8	45

b.

Clone	b^n	Intercept a^n	y^n where $x^n=7$	$b[^I]$	Intercept $a[^I]$	$b[^T]$	Intercept $a[^T]$
469	0.20**	1.74	3.14	0.71*	0.01	0.88**	−0.46
23	0.50**	0.94	4.44	0.99*	−0.83	1.12**	−1.04
26	0.67**	0.29	4.98	0.93*	−0.43	2.04*	−2.16
339	0.57**	0.91	4.90	1.57**	−0.57	2.45**	−1.78
12	0.69**	0.49	5.32	1.92**	−4.16	2.37**	−3.74
9	0.69**	0.94	5.77	0.97**	−0.41	2.33**	−2.65
64	0.67**	0.26	4.95	0.69*	0.90	1.25*	0.01

* Significant at 5% level
** Significant at 1% level ("F" test)

clone 9 were obtained by direct calculation from the formula shown in Figure 1. b^n was calculated from the individual numbers immediately following each "index number" and the respective "index number" of each lateral flowering shoot. Also shown are values of the intercept a^n (i.e. y^n where $x^n=0$), and y^n where $x^n=7$—since this was the maximum value of x^n common to all clones.

At the stage of growth and development in which these data were recorded, further formation of buds and inflorescences was yet to occur—particularly in clones of the late-flowering growth types. In general, however, the largest numbers of buds, non-flowering and flowering shoots were produced on stems of the late-flowering clones, as shown in Table 1.

The linear regression b^n was highly significant for each clone. Therefore, *the number of nodes on any lateral flowering shoot was positively correlated with, and linearly related to, the number of the node at which each was borne.* All values of b^n were between 0.20 (early-flowering clone 469) and 0.69 (later flowering clones 12 and 9). The slopes of y^n plotted against x^n would be steepest in the later flowering clones and least steep in the early flowering clones, i.e. in the former clones y^n approximated most closely to 7 (where $x^n = 7$).

(2). Theoretically, the number of inflorescences at each successive primary node, [I], would be related exponentially to primary node number:

$\log [I] = a - bx \ldots x =$ primary node number.

Contrary to theoretical expectations, however, it was found that the data in Table 1 did not show an exponential relationship but did conform to the linear regression formula:

$y[^I] = a[^I] + b[^I]\, x[^I] \ldots y[^I]$ = number of inflorescences at each successive primary node.

$x[^I]$ = primary node number at which lateral is borne.

The linear regression coefficient $b[^I]$ and the intercept $a[^I]$ were calculated from the successive number of symbols for inflorescences separated by single dots, and the respective primary node numbers.

$b[^I]$ was significant for all clones and was highly significant for clones 339, 12 and 9. The latter clones possessed the highest number of inflorescences upon a stem. The data, therefore, showed that *the number of inflorescences at each successive primary node was linearly related to primary node number.*

(3). Theoretically, the number of buds, non-flowering and flowering shoots at each successive primary node, [T], would be related exponentially to primary node number. But, similarly to [I] above, the data in Table 1 did not show an exponential relationship but did conform to the linear regression formula:

$y[^T] = a[^T] + b[^T]\, x[^T] \ldots y[^T]$ = number of buds, non-flowering and flowering shoots at each successive primary node.

$x[^T]$ = primary node number at which lateral is borne.

The linear regression coefficient $b[^T]$ and the intercept $a[^T]$ were calculated from the successive number of symbols for buds, non-flowering and flowering shoots separated by single dots, and the respective primary node numbers.

$b[^T]$ was significant for all clones and was highly significant for clones 469, 23, 339, 12 and 9. The data therefore showed *that the number of buds, non-flowering and flowering shoots was linearly related to primary node number.*

(c) *Influence of Growth Regulator Treatments*

Results for entire primary stems of plants of clone sprayed with growth regulators are shown in Tables 2 and 3. Analysis was by the same procedures as for Table 1.

The following results (Table 2) are from data recorded on August 29, 1955 for treatments of water, 50 IAA, 50 NAA, TIBA 100, and MH 800:

(1) (*a*). The linear regression coefficient b^n was highly significant for each treatment. All values were between 0.70 and 0.94. The slopes of y^n plotted against x^n would be steepest for 50 IAA and progressively less for TIBA 100, water, MH 800, and 50 IAA.

(*b*). Values of b^n for the first, second, and third order lateral shoots, i.e. b^{n1}, b^{n2}, and b^{n3} respectively, were highly significant for each treatment except b^{n3} of MH 800. In general, the values of b^{n1} and b^{n2} were higher than b^{n3}. Also, in general, the values of y^{n1} (where $x = 5$) approximated more closely to 5 than y^{n2}, while y^{n3} approximated least closely.

The following results (Table 3) are from the same data as for Table 2 and also from data recorded September 23, 1955, for treatments of water, NAA 50, TIBA 25, TIBA 100.

(2) and (3). The linear regressions $b[^I]$ and $b[^T]$ were highly significant for all treatments. Therefore, the normal linear relationships of either [I] or [T] to primary node number (Table 1) were not changed by the growth regulator treatments. In general, however, the growth regulators increased the number of buds, shoots and inflorescences formed upon a stem. TIBA decreased the rate of stem elongation of primary buds and, as a result, there were fewer well developed stems with the TIBA treatments. TIBA did, however, increase the number of crown buds and also the number of lateral buds formed on well developed stems. NAA 50 resulted in the highest increase in number of lateral buds formed during the experiment. Cumming (5) found that TIBA spray treatment significantly increased crown yield but significantly decreased stem and seed yield of spaced red clover plants under field conditions; IAA spray treatment significantly increased seed yield.

TABLE 2.—LINEAR REGRESSIONS (b^n) OF NUMBER OF NODES ON FLOWERING SHOOTS ON NODE NUMBER AT WHICH BORNE. FIELD PLANTS SPRAYED WITH GROWTH REGULATORS. CLONE 9. DATA RECORDED AUGUST 29, 1955.

Treatment	All lateral flowering shoots			Order of lateral flowering shoots								
				First			Second			Third		
	b^n	Intercept a^n	y^n where $x^n=5$	b^{n1}	Intercept a^{n1}	y^{n1} where $x^{n1}=5$	b^{n2}	Intercept a^{n2}	y^{n2} where $x^{n2}=5$	b^{n2}	Intercept a^{n2}	y^{n2} where $x^{n2}=5$
Water	0.74**	0.47	4.22	0.74**	0.93	4.63	1.0**	−1.0	4.00	0.64**	0.94	4.14
50 IAA	0.94**	0.14	4.84	0.77**	0.86	4.71	0.75**	1.15	4.90	0.69**	0.67	4.12
50 NAA	0.69**	0.72	4.17	0.68**	1.60	5.00	0.61**	1.04	4.05	0.66**	0.68	3.98
TIBA 100	0.82**	0.12	4.22	0.71**	1.74	5.29	0.76**	0.65	4.45	0.73**	0.13	3.78
MH 800	0.70**	0.72	4.22	0.70**	0.80	4.30	0.70**	0.77	4.27	0.30	1.97	3.47

* Significant at 5% level
** Significant at 1% level. ("F" test)

TABLE 3.—LINEAR REGRESSIONS: $b[^I]$ – TOTAL NUMBER OF INFLORESCENCES AT EACH SUCCESSIVE NODE, [I], ON PRIMARY NODE NUMBER AT WHICH BORNE; $b[^T]$ – TOTAL NUMBER OF BUDS, NON-FLOWERING AND FLOWERING SHOOTS AT EACH SUCCESSIVE PRIMARY NODE, [T], ON PRIMARY NODE NUMBER AT WHICH BORNE. FIELD PLANTS SPRAYED WITH GROWTH REGULATORS. CLONE 9. DATA RECORDED ON AUGUST 29 AND SEPTEMBER 23, 1955.

Node number	Aug. 29, 1955 Growth regulator treatments										Sept. 23, 1955 Growth regulator treatments							
	Water		50 IAA		50 NAA		TIBA 100		MH 800		Water		NAA 50		TIBA 25		TIBA 100	
	[I]	[T]	[I]	[T]	[I]	[T]	[I]	[T]	[I]	[T]	[I]	[T]	[I]	[T]	[I]	[T]	[I]	[T]
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	2	3	5	2	3	1	3	4	4	5	7	2	2	3	3
3	3	3	4	5	5	12	4	7	3	6	9	11	14	26	5	8	4	6
4	4	6	5	8	11	16	8	16	3	8	14	33	17	34	9	11	6	10
5	5	8	6	8	14	27	11	19	4	11	24	38	36	62	9	11	11	17
6	7	10	8	14	17	28	14	23	6	13	27	38	43	60	13	14	14	22
7	8	15	10	16	22	42	16	33	7	17	43	67	65	93	18	21	14	24
8	13	22	8	18	28	43	30	59	10	23					31	43	23	41
9	10	22	17	34					7	26					28	35	26	53
10									14	29								
Total	52	88	60	106	101	174	86	161	56	137	122	192	181	283	116	146	102	177
b	1.42**	2.92**	1.67**	3.47**	3.85**	6.4**	3.64**	7.23**	1.25**	3.19**	6.68**	10.46**	10.36**	14.93**	3.75**	4.8**	3.13**	6.17**
Intercept a	−1.32	−4.82	−1.68	−5.57	−4.70	−7.05	−5.63	−12.41	−1.28	−3.85	−9.29	−15.84	−15.58	−19.29	−5.86	−7.78	−4.32	−11.18

* Significant at 5% level
** Significant at 1% level. ("F" test)

For explanation of growth regulator treatments see text

(d) *Conclusions*

From the above theoretical postulations regression values of b^n less than 1 would be expected if the lateral apices grew, i.e. produced nodes, at a slower rate than their parental apices. This could entail relatively slower growth of succeeding orders of lateral shoots, and could be expected to result from the phenomenon of apical dominance. Transmission of a systemic non-polar flowering influence would terminate vegetative growth and thus confer determinacy on deviations from the theoretical pattern existing at any particular stage.

Almost all of the observed results show that there was a significant degree of conformity to the "expected node numbers for flowering". In general, the clones with the least pronounced apical dominance were the ones from which the highest regression values b^n were obtained and the ones with values of y^n approaching most closely to x^n. This was shown to the greatest extent in some late-flowering clones.

The general effect of growth regulators in the experiments recorded above was to reduce the amount of apical dominance. Cumming (5) found in red clover that the influence of auxin varies according to day-length; non-vernalized plants watered with IAA solutions showed decreased apical dominance under short and intermediate daylengths (8- and 16-hour daylengths respectively) but increased apical dominance under continuous light. Thus, auxin and anti-auxin (TIBA) can both, under certain conditions, bring about increased lateral bud formation. The non-vernalized plants under field conditions at Macdonald College showed an intermediate daylength type of reaction to auxin; this was consistent with the prevailing daylength conditions. The results are also consistent with the known effects of auxins and anti-auxins. Apical dominance and flowering node numbers can be increased or decreased to some extent without influencing flowering in a more qualitative manner. It is noteworthy that after spraying with 50 IAA the value of b^n (0.94) approached closely to the theoretical, and where $x^n = 5$, $y^n = 4.84$.

Conformity to the "expected node numbers for flowering" was least on the higher order lateral shoots. Also, with increase in values of x^n, values of y^n approximated less closely. The key in Figure 1 illustrates the general observation that, with low values of x^n, y^n was generally equal to or more than x^n, whereas, with higher values of x^n, y^n was equal to or less than x^n. These effects are consistent with the postulation that apical dominance could result in suppression, or at least a decrease in rate of growth, of lateral shoots—as a function of increase in their order and also in node number at which they are borne. Floral initiation would then confer determinacy on such deviations.

On this basis, the linear regression of [I] and [T] on primary node number can also be interpreted. If it is assumed that there is an additive effect in apical dominance as a function of (1), the total number of higher order laterals arising from each primary node, and (2), the number of the node subtending each lateral, then the relative suppression of lateral buds and shoots would be more pronounced with increase in the number of the primary node.

In this respect it can often be noted that only the first few laterals below the flowering node on a primary stem may develop on plants grown either in the greenhouse or under conditions of close field planting. These laterals are often restricted to the first and second orders [see figures in (5), (10)]. This extreme is consistent with the type of effects just discussed.

Onset of the Second Cycle of Vegetative Growth

(a) *Precocious Onset*

Growth occurring after August 29, 1955, indicated that MH 800, which included two sprays at 800 p.p.m., had a pronounced influence in morphogenesis. This was the maximum concentration of MH employed; lower concentrations applied earlier in the season were found to have little visible effect and the higher concentration was therefore tested. Figure 2 illustrates entire stems taken from plants of clone 9 on October 22, 1955. Fully developed inflorescences were absent from plants after spraying with MH 800. A much larger number of vegetative higher order lateral buds were developed as compared to the controls; this was because MH had stopped inflorescence formation and seed set but hastened onset of a further cycle of vegetative growth. Leaves produced after MH 800 treatment were at first smaller in size, more irregular in outline, thicker and more brittle than normal; they were pale bluish-green in colour. Stems of treated plants were more brittle and succulent but their length of life was greater. The rooting and later growth of propagules taken from plants subjected to MH 800 were quite normal; a normal potentiality for indeterminate vegetative growth was shown.

Figure 3 illustrates a further example of precocious onset of vegetative growth on stems that had initiated floral primordia. Normally, in red clover, further vegetative growth upon a stem can follow after maturation of the first cycle of inflorescences on that stem (if the latter remains alive). The plant shown in Figure 3 was still under photoinductive cycles when photographed on August 22, 1955; it was present in the breeding nursery at Macdonald College and had shown progressive symptoms of virus disease. Symptoms included abortion of inflorescences, thinning and yellowing of stems and leaves; these were found to occur quite frequently under field conditions. A range of effects similar to those arising from virus infection has also been produced by spraying plants with IAA at concentrations high enough to cause abortion of inflorescences under photoinductive cycles (5).

(b) *Normal Onset*

Other studies were conducted to further elucidate the normal pattern of growth and development following floral initiation. Plants of clone 44 were maintained under photoinductive cycles in the field from June 1 to September 3, 1955. They were then transferred to greenhouse conditions, without removing their flowering and post-flowering stems. Equal numbers of plants were placed under LD and SD of 16-hour and less than 14-hour daylengths, respectively. On December 6, 1955, a count of nodes was made on stems which had previously flowered but remained with

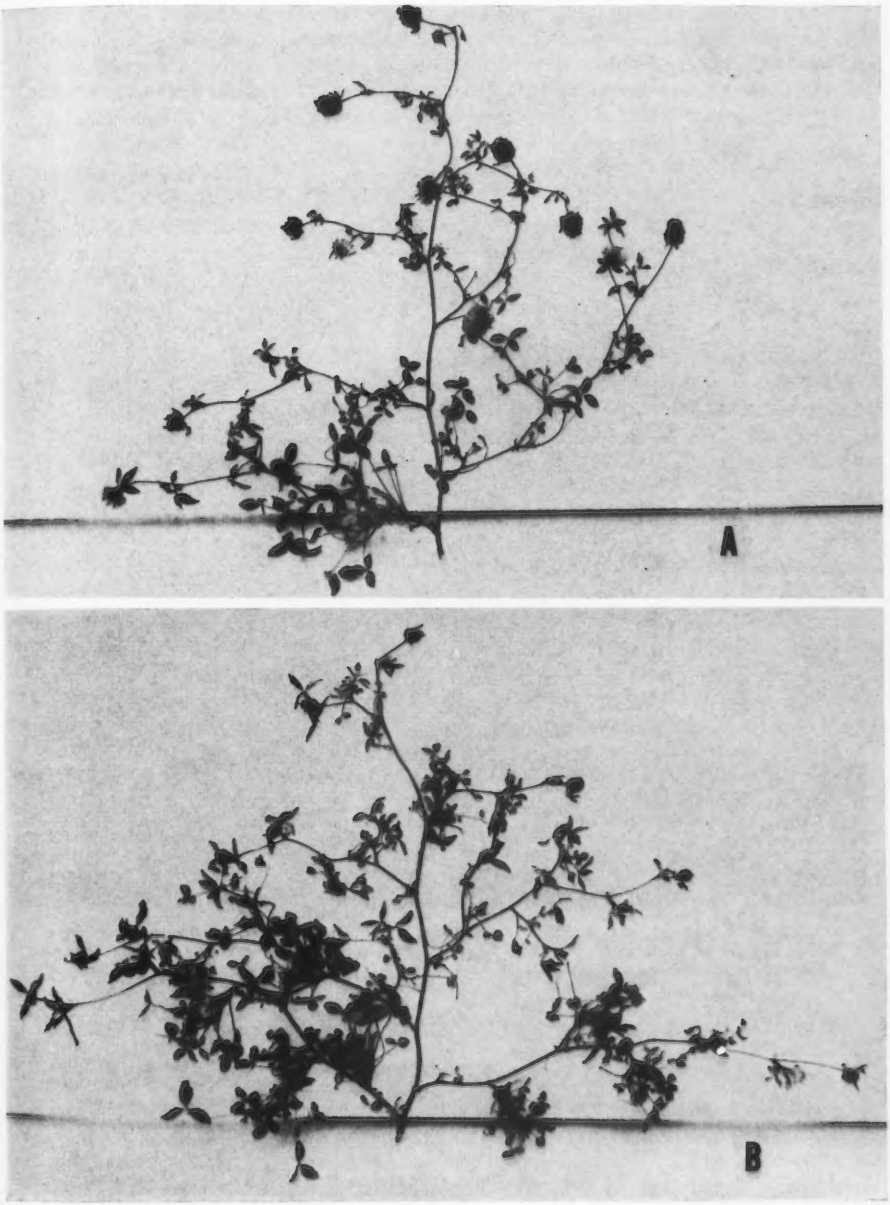

FIGURE 2. Clone 9. Entire stem from plants. (Photographed October 20, 1955).
A. Control spray (0) *B*. Sprayed with MH 800

FIGURE 3. Precocious onset of vegetative lateral bud formation under long days following floral initiation. Spaced plant which had shown progressive signs of virus disease; from breeding nursery, Macdonald College. (Photographed August 22, 1955).

some lateral buds or shoots still alive under the prolonged LD. The following values are representative of 6 plants under 16-hour daylengths. The first value of each of the paired figures represents the node number of the stem at which the higher order lateral bud was produced, the second value represents the observed number of leaves on this higher order lateral bud or shoot:

4/7, 2/8, 3/9, 2/8, 2/7, 2/9, 3/8, 3/11, 3/9, 3/9, 2/7, 3/7, 3/7, 2/7, 2/7, 2/8.

No inflorescences were visible on these laterals, despite the imposition of continuous photoinductive cycles from a time prior to floral initiation. The leaf numbers far exceeded the values expected if inflorescences had been initiated at higher order lateral shoots according to the "expected node numbers for flowering". The linear regression of observed number of leaves on the lateral buds, on respective node numbers at which each was borne, was not significant. Thus, even under normally photoinductive cycles, flowering at the lower order lateral shoot apices was followed by vegetative growth at the higher order apices. However, flowering was apparent on some young primary stems of plants under LD. This suggests that some stimulus for flowering was present within the plants—even though higher order lateral buds upon stems previously induced to flower were incapable of responding to it during this time.

A larger number of lateral buds in active growth were present under SD but these were at the stage of indeterminate vegetative growth. Flowering did not occur on the young primary shoots.

Similar indications were noted under field conditions on plants of the early-flowering clone 26. The examples below are taken from data recorded when the daylength was still above the critical photoperiod. Emerged leaf number was used for estimating minimum node number on those buds and non-flowering shoots showing appreciable growth. Leaf numbers for buds or shoots exceeding the "expected node numbers for floral initiation" are shown underlined; inflorescences were not found on these.

Clone 26 (a). First order lateral shoot—.M^{5}5:M^{2}2:.Ab^{2}2Ab^{3}3::B^{2}B^{3}:M^{3}2:.M^{2}2::Ab^{2}2B^{3}:.Ab^{3}3::*B^{2}5B^{3}5*:M^{4}3

(b). First order lateral shoot—.M^{6}5:*B^{1}3*Ab^{2}2M^{3}3:.*E^{2}5*::B^{3}B^{4}E^{5}4B^{6}:.B^{3}:M^{4}3:.*E^{2}7*::B^{5}B^{6}E^{7}B^{8}:.*E^{3}7*::B^{4}B^{5}B^{6}B^{7}B^{8}:B^{5}

(c). Third order lateral shoot—:.M^{2}2::M^{2}2Ab^{3}3::.*B^{2}4B^{3}4*:.Ab^{4}3::*B^{2}5*B^{4}B^{5}:.B^{3}5B^{5}

DISCUSSION

In terms of growth and development upon any one stem, a relative estimate of potentiality in different genotypes grown under any given set of conditions can be obtained by use of the key described. The influence of growth regulators or other treatments can also be evaluated. Some estimate of the potential range of phenotypic variability of any particular genotype can then be obtained. These considerations are important in the evaluation of both inherent and environmental factors controlling

growth and development, and therefore yield, of different plant parts in either crop or weed species. For example, the node number prior to flowering was less in the early- as compared to late-flowering growth types. The potential for total number of inflorescences and, ultimately, number of seeds that could be produced on a primary stem, was, therefore, lower in the early-flowering types. Even under field conditions the potentiality for lateral bud formation was by no means realized in the control spaced plants—as shown for example by the marked increase with spray treatment NAA 50 (Table 3).

Attainment of "ripeness-to-flower" at certain apices coincident with the production of a florigenic stimulus offers an explanation for the observed pattern of floral initiation in the "first cycle of growth". Onset of the "second cycle of vegetative growth" could result from one or a combination of causes, for example, a lack of photoperiodic response by the leaves, inability of the stems to conduct a florigenic stimulus, destruction or loss of florigenic stimulus, or incapacity of apices in the "second cycle of vegetative growth" to attain "ripeness-to-flower". Grafting experiments or the use of gibblerellins [which have florigenic properties in some species—see (25) for lit. cit.] offer possible means of solving such problems.

With cessation of floral initiation and onset of the "second cycle of vegetative growth" succeeding order lateral shoot node numbers exceeded the "expected node numbers for flowering". Indeterminate vegetative growth was, therefore, superimposed on the determinacy of flowering. Buds on propagules taken from the "second cycle of vegetative growth" have required further periods of photoinductive cycles to effect initiation of floral primordia, even though the parent plants had been maintained under photoinductive cycles (5).

It is interesting to contrast the sequence of vegetative growth and flowering upon primary stems in red clover with, on the one hand, that of normally strict annuals—in which one cycle of flowering terminates the life of the stem, and on the other, that of woody perennial plants—in which secondary growth and a series of cycles of vegetative growth and flowering enable the plant to increase its stem size and also to reproduce in each year. Red clover stems illustrate a part of the range between these two extremes; the extent of this range is a function of genotype and environmental conditions.

Growth and development of any stem can be considered as potentially a series of successive and repetitive cycles of vegetative growth followed by flowering, although the original sequence of floral initiation can terminate vegetative bud initiation upon the stem. Expression of potentiality will be governed by the amount and rate of vegetative growth and flowering in any particular cycle; at the same time it is dependent on ability of the stems to support growth of the laterals.

It has been shown that MH, which brought about abortion of inflorescences, hastened onset of the "second cycle of vegetative growth" (Figure 3). A further effect was to increase the longevity of stems on treated as compared to control plants. A larger number of buds in the "second cycle of vegetative growth" can, therefore, be obtained earlier through cessation of growth and differentiation at the floral apices of the

first cycle. The use of MH to hasten onset of this "second cycle of vegetative growth" has shown considerable promise as a means of increasing the number of "leaf-bud" propagules suitable for vegetative propagation (5). Gifford (6) has shown that in barley the initial and deleterious effects of MH are upon meristematic tissues and tissues that are undergoing differentiation. Wittwer (29) found that spraying "bolting" sugar beets with 1000-5000 p.p.m. solutions of MH stopped further flower development and caused reversion of plants to an apparently vegetative state.

It is of added interest that precocious onset of the "second cycle of vegetative growth" under photoinductive cycles has been observed as a secondary effect of virus disease (Figure 3). Initiation of floral primordia can occur in plants of red clover showing virus symptoms but these primordia may not develop fully and there is then a hastening in onset of the "second cycle of vegetative growth". A similarity between these symptoms and application of IAA is indirect evidence for an interaction between virus and endogenous auxin production. Nickell (21) has reviewed some indirect evidence for interaction of virus and auxin in other plant species. Jones (12) has found higher auxin levels in virus infected tobacco and tomato plants than in healthy plants.

The extent to which the potentiality of any particular genotype can be more nearly expressed or suppressed, by manipulation of environmental and other conditions, must be recognized as a fundamental question. Further knowledge in this respect offers the promise of decisive advances in agronomic techniques.

ACKNOWLEDGEMENTS

The author has pleasure in thanking those who extended help during the course of these investigations, in particular, the Goldsmiths' Company of Great Britain for the award of a Travelling Scholarship for the years 1952-1954; the Province of Quebec Agricultural Research Council for providing financial assistance; L. C. Raymond, formerly Chairman, and H. A. Steppler, present Chairman of the Department of Agronomy, Macdonald College, McGill University, for the facilities granted and for their helpful advice.

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VARIATION AMONG ROWS IN POWER-SEEDED CEREAL VARIETY TRIALS[1]

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[Received for publication June 5, 1958]

ABSTRACT

Variations in survival of winter wheat and in yields of winter wheat, spring wheat, and barley were obtained among rows of power-seeded variety trials where there was differential packing by tractor wheels. This variation makes variety comparisons difficult and introduces bias in single-row-plot and two-row-plot trials. A power seeder with four drive-wheels giving uniform packing of all rows greatly reduced the variation in yield among rows.

INTRODUCTION

Four-row power seeders are used commonly to reduce time and labour involved in seeding experimental cereal plots. A number of different types of seeders have been described (1, 2, 3,). Two different types of power seeder used at the Experimental Farm, Lethbridge, Alberta, are shown in Figures 1 and 2. With most of these machines all of the four rows seeded are not treated similarly as far as packing by the tractor or drive-wheels is concerned. This is not a serious problem where the machines are used for seeding four-row plots because any resulting variation is within plots. However, these machines also are used extensively to seed two-row-plot and single-row-plot tests, the latter usually being selection nurseries. In these cases the differential packing may result in serious bias in making comparisons.

Data are given herein that show these errors and illustrate principles that should be considered in the design and construction of improved power seeders.

EFFECT ON WINTER WHEAT

In the fall of 1950, a replicated test of winter wheat varieties in four-row plots with 1-foot spacing between rows was seeded on irrigated land with the power seeder shown in Figure 1. With 1-foot row spacing and a width of 54 inches between centres of the tractor wheels, the first row of each plot was seeded into soil packed by a tractor wheel while seeding the previous adjacent plot, and the last row was packed by a tractor wheel while seeding the next adjacent plot. Since the soil was comparatively loose, the first row was sown shallower than the other three.

The effect of this differential packing on survival in the spring of 1951 is shown in Figure 3. In every plot, survival was much greater in the first row than in the other three rows.

Similar but less severe differential winterkilling occurred in 1952 and yield data were obtained from individual rows of one four-row-plot test of 13 varieties in four replicates. This test also was seeded with the power

[1] Joint contribution from the Divisions of Cereal Crops (Contribution No. 232), and Illustration Stations (Contribution No. 107), Experimental Farms Service, Canada Department of Agriculture.

FIGURE 1. Tractor-drawn power seeder used at Lethbridge, Alta.

seeder shown in Figure 1 but with 9-inch spacing between rows. With this spacing, the first two rows of each four-row plot were seeded on the edges of a tractor-wheel mark and consequently into packed soil, and the last two rows were packed to some extent by a tractor wheel after seeding. The average yield from Row 2 of all plots was 25.8 bushels per acre, while that from Row 3 was only 12.5 bushels per acre.

Similarly in 1956 major differences in survival and yield of winter wheat were obtained among different drill-rows of each power-seeded plot. These plots had been seeded in the bottom of shallow furrows because of dry soil conditions in the fall. The spacing between rows was 12 inches and that between tractor-wheel centres was 48 inches. Thus the first and fourth drill-row were next to a tractor-wheel mark. The tractor wheels flattened out the protective furrow ridge beside these rows and tended to partly fill in the fourth furrow. Thus seed in Row 4 was covered more deeply than in the other three rows. This resulted in differential spring survival and yield among drill rows. The average survival and yield obtained from each drill row of 100 four-row plots of winter wheat were:

	Drill Row			
	1	2	3	4
Survival (per cent)	34.4	40.9	39.4	11.7
Yield (bu./acre)	43.5	42.1	44.4	22.2

FIGURE 2. Power seeder with four drive-wheels used at Lethbridge, Alta.

Survival and yield were much lower in Row 4 than in the other three rows. Survival in Row 1 also was lower than in Rows 2 and 3. This difference in survival most likely resulted from the flattening of the protective ridge beside Rows 1 and 4 and the partial filling of furrow 4. The difficulty in making valid comparisons for survival and yield between varieties and lines of winter wheat seeded in single-row plots with this seeder arrangement is apparent.

Figure 4 shows survival in the spring of 1956 in a two-row-plot test of winter wheat sown in a similar manner to the one described above. The difficulty in making unbiased comparisons in this test is obvious.

EFFECT ON SPRING WHEAT AND BARLEY

Because of the marked variation obtained among rows in winter wheat tests it seemed desirable to investigate the possible occurrence of similar variation in power-seeded spring wheat and barley tests, even though variation usually was not visually apparent. Accordingly, in 1956, yield data were obtained for individual rows of four-row-plot variety tests of spring wheat and barley seeded in rod-rows with the seeder shown in Figure 1.

The barley test consisted of 16 varieties with four replicates. The spacing between rows was 9 inches and that between the tractor-wheel centres was 54 inches. The average yield obtained from each of the seeder drill-rows was:

	Drill Row			
	1	2	3	4
Yield (bu./acre)	75.0	62.2	53.3	67.1

There was a significant difference ($P<.01$) in yield between rows and a significant variety x row interaction ($P<.05$). It is evident that, if a single-row-plot test had been seeded in a similar manner, there would have been considerable bias in the results obtained.

In order to simulate a two-row-plot test, the yields of Rows 1 and 2, and those of 3 and 4, were combined. The average yield obtained from the first two rows was 68.6 bushels per acre and from the last two was 60.3 bushels per acre. The difference between the two was significant ($P<.01$) and, again, if a two-row-plot test had been seeded in a similar manner, there would have been considerable bias in the results.

The wheat test consisted of six varieties in four replicates seeded with the same seeder as the barley test. The difference in yield between drill-rows was significant ($P<.01$). The yields obtained are given below:

	Drill Row			
	1	2	3	4
Yield (bu./acre)	43.6	42.5	39.9	38.6

The yields of Rows 1 and 2 and of Rows 3 and 4, combined to simulate a two-row-plot yield test, were 43.0 and 39.3 bushels per acre respectively. Although these values were not significantly different ($P>.05$) the trend was similar to that obtained in the barley test where the first two rows gave the highest combined yield. Again it was evident that, if either a single-row-plot or a two-row-plot test had been seeded in a similar manner, there would have been considerable bias in any variety comparisons made.

EFFECT OF VARIOUS COMBINATIONS OF ROW PACKING ON SPRING WHEAT AND BARLEY

In the spring of 1957 the power seeder shown in Figure 2 was available for seeding. This machine has four drive-wheels spaced to pack the soil directly in front of the four drill-rows and either the two inner or the two outer drive-wheels may be removed independently.

This machine was used to seed a split-plot test involving Rescue wheat and Vantage barley with three combinations of row packing as follows: (1) Wheel packing in front of all four rows; (2) Packing in front of the two centre rows only; and (3) Packing in front of the two outer rows only. There were nine replicates. Rows were harvested individually and the average yields obtained are given in Table 1.

FIGURE 3. Differential spring stands in winter wheat in 1951 resulting from seeding the first row of each four-row plot into soil packed by a tractor wheel.

FIGURE 4. Differential spring stands in 1956 in a winter wheat test seeded in furrows where Rows 1 and 4 were affected by tractor wheels.

TABLE 1.—YIELDS IN BUSHELS PER ACRE OF INDIVIDUAL ROWS OF WHEAT AND BARLEY SEEDED WITH DIFFERENT COMBINATIONS OF ROW PACKING

Crop and rows packed	Row of seeder			
	1	2	3	4
Barley				
All four	29.2	29.7	30.4	30.0
Rows 1 and 4 only	34.0	28.0	26.5	36.7
Rows 2 and 3 only	23.7	32.6	35.0	27.1
Wheat				
All four	13.0	12.9	13.8	11.9
Rows 1 and 4 only	14.6	12.1	12.2	12.2
Rows 2 and 3 only	12.3	13.0	12.6	11.3

For barley, there was no significant difference in yield between rows when all four rows were seeded into a wheel mark. However, when only the two outer rows were sown into the wheel marks the yields obtained were significantly higher ($P < .01$) than those from the inner two. Similarly, when only the two inner rows were seeded into the wheel marks, significantly higher ($P < .01$) yields were obtained from them than from the outer two.

For wheat, there was no significant difference in yield between rows for any of the treatments. However, the trend was similar to that obtained for barley.

DISCUSSION

The data presented herein show that, where there is differential packing among rows of power-seeded variety trials, considerable variation may be expected among rows for survival of winter wheat, and for yields of winter wheat, spring wheat, and barley. The effect was less pronounced for spring wheat than for either of the other two crops in the years when data were recorded. The variation among rows may be attributed to either differences in soil compaction or depth of seeding, or a combination of the two. Where power seeders are used for seeding two-row or single-row-plot tests, this variation will result in serious bias in making variety comparisons.

The variation among rows for spring wheat and barley was reduced greatly by using a power seeder with four drive-wheels that packed the soil directly in front of the seeder disks (Figure 2). This machine might be improved further by replacing the two outrigger wheels, which support the seeder, by four press wheels following directly behind the seeder disks. Thus, all four rows would be treated similarly both before and after seeding and much of the variation among rows removed. The principle of uniform packing of rows should be incorporated into the design and construction of a good power seeder.

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EFFECT OF INSECTICIDES AND HERBICIDES APPLIED TO SOIL ON THE DEVELOPMENT OF PLANT DISEASES

II. EARLY BLIGHT AND FUSARIUM WILT OF TOMATO[1]

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[Received for publication June 16, 1958]

ABSTRACT

The effects of several insecticides and herbicides on the development of early blight and Fusarium wilt of tomato were investigated. Young tomato plants were grown in sand to which solutions or suspensions of these chemicals were applied repeatedly before the foliage was inoculated with spores of *Alternaria solani* and either before or after the roots were inoculated with spores of *Fusarium oxysporum* f. *lycopersici*. On the basis of lesion counts, increased early blight development resulted from applications of lindane, 2,4-D, and isodrin, and decreased disease from endrin, MH, NPA, dieldrin, IPC, dalapon, demeton and aldrin. Lindane, isodrin, and dalapon increased the severity of Fusarium wilt whereas endrin, aldrin, TCA, DDT, and dinoseb reduced it. 2,4-D and MH affected wilt development in a susceptible and a resistant variety in different ways according to the time of application in relation to inoculation, but they did not alter the reaction of an immune variety.

INTRODUCTION

The effects of soil applications of certain insecticides and herbicides on the development of the seedling disease of barley caused by *Helminthosporium sativum* P. K. and B. were reported previously (9). The present paper concerns the effects of these chemicals on the development of two diseases of tomato plants: a foliage disease, early blight, caused by *Alternaria solani* (Ell. & Mart.) Jones & Grout; and a vascular wilt, caused by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans.

MATERIALS AND METHODS

Tomato plants of the variety John Baer were used in all tests except where otherwise specified. Seedlings started in flats of sand were transplanted into waxed paper cartons filled with sand. The plants were supplied with water and nutrient solution as required.

The chemicals tested included all of those listed in the previous report (9) with the exception of IPX and the addition of demeton (*O*-*O*diethyl *O*-2-(ethylthio)ethyl phosphorothioate. A logarithmic dosage series of each chemical (e.g. 6.25, 12.5, 25, 50 and 100 p.p.m.) was prepared either as an aqueous solution or as a suspension made by injecting 1 part of an acetone solution of the chemical into 99 parts of water. A measured volume of the chemical preparation was applied to the sand daily over a period of 10 days starting when the plants were approximately 5 inches high. Control plants received an equivalent volume of water or 1 per cent acetone solution at the same time.

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[2] Senior Plant Pathologist.

Plant heights were measured before and after treatments and again at the end of each test to determine the effect of the chemicals on plant growth. Leaves and roots were also examined for manifestations of phytotoxicity.

Early blight inoculations were made on the day following the final application of chemical. Eight replicate plants from each treatment were placed on a turntable and sprayed with a measured volume of a suspension of Alternaria spores. The plants were kept for 2 days in a lighted incubation chamber with high relative humidity, then returned to the greenhouse. When disease symptoms had developed sufficiently the number of lesions on each of two or more leaves at corresponding positions on the plants were counted. The number of leaves which had abscissed was also recorded.

Fusarium wilt inoculations were made by washing the roots of young tomato plants free of sand, immersing them in a suspension of microconidia produced in shake-culture in Czapek's liquid medium, and replanting them in cartons of sand. In most of the tests one set of plants was inoculated the day before the first application of chemical and a parallel set the day after the final application. Roots of non-inoculated control plants were washed and replanted at the same times as the inoculated plants. After inoculation the plants in cartons were kept on a bed of gravel maintained at 28° C., the optimum temperature for the development of wilt symptoms, by means of a buried heating cable with thermostatic control.

The degree of disease development was assessed on the basis of both external and internal symptoms. The degree of visible wilting of each plant was rated on a scale of 0 to 100 per cent by the method of Gallegly and Walker (5). The extent of vascular browning observed in longitudinal stem sections was rated from 0 to 4 as described by Scheffer and Walker (11). More consistent results were obtained by the former method and these data are used for comparative purposes in this report.

EXPERIMENTAL RESULTS

Effects of Chemicals on the Host

The plant height measurements indicated that most of the chemicals caused some stunting of the tomato plants, particularly at the higher dosage rates. Lindane was phytotoxic at concentrations above 6.25 p.p.m. retarding the development of stems, leaves, and roots. Monuron caused severe stunting and leaf scorching at 0.05 p.p.m. and was lethal at higher concentrations. Leaf scorching also resulted from the higher dosages of schradan and demeton. On the other hand a few chemicals appeared to stimulate plant growth. Increased plant heights followed applications of aldrin or DDT (6.25 to 100 p.p.m.), endrin (6.25 to 50 p.p.m.), or dalapon (0.25 to 4 p.p.m.).

Effects of Chemicals on Early Blight Development

As a basis of comparison of the effects of various chemicals on early blight development, the percentage increase or decrease in number of lesions per leaf on treated plants over the number developed on control

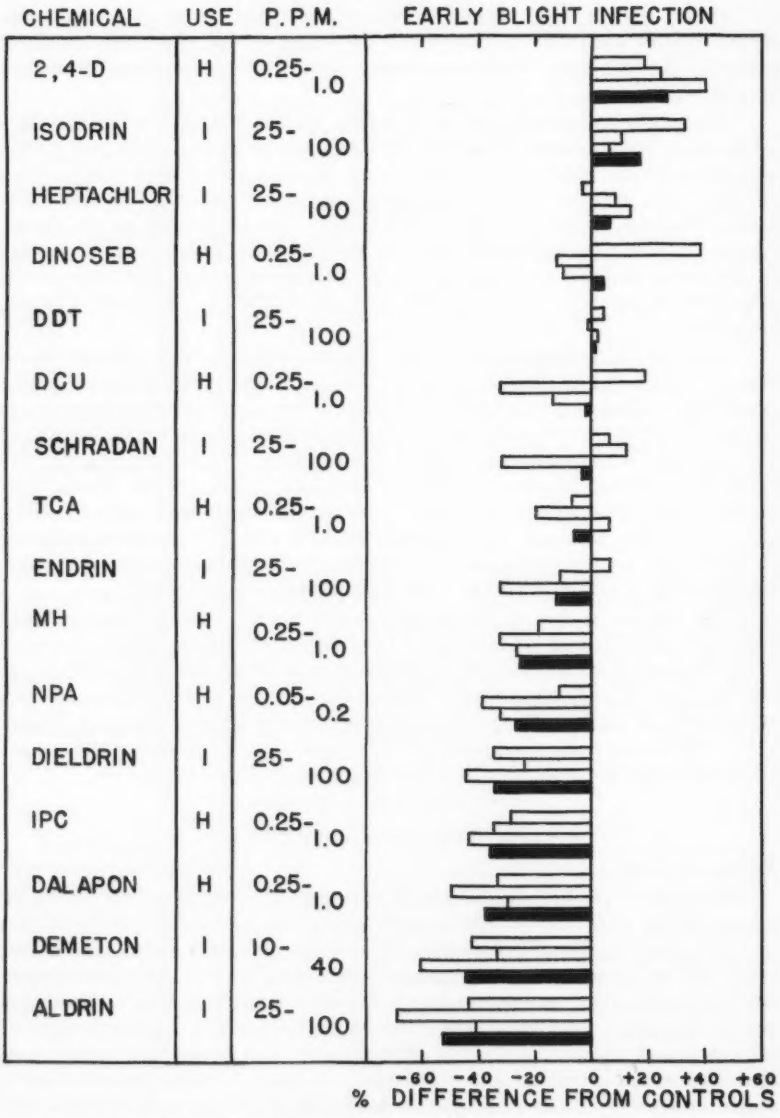

FIGURE 1. Effects of various chemicals applied to sand before inoculation of tomato foliage on development of early blight. *White bars* from top to bottom in each group represent percentage difference in numbers of lesions per leaf between treated and untreated plants with increasing dosages of chemical; *black bar* represents mean effect of all dosages in the logarithmic series.
I = insecticide, H = herbicide.

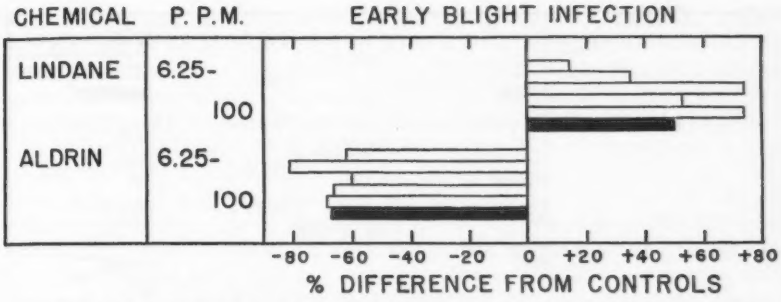

FIGURE 2. Effects of two insecticides applied to sand before inoculation of tomato foliage on development of early blight. *White bars* from top to bottom in each group represent percentage difference in number of lesions per leaf between treated and untreated plants with increasing dosages of chemical; *black bar* represents mean effect of all dosages in the logarithmic series.

plants was computed. Figure 1 shows the relative amounts of disease that developed following treatment with 16 chemicals which are arranged in the order of their effect from maximum increase to maximum decrease. Definite evidence of increased disease resulting from chemical treatment was obtained with 2,4-D. Isodrin also increased the lesion counts, although the effect did not vary directly with the dosage. The effects of heptachlor, dinoseb, DDT, DCU, schradan, and TCA are not considered significant because no consistent change in numbers of lesions followed their application. Applications of endrin, MH, NPA, dieldrin, IPC, dalapon, demeton, and aldrin consistently reduced the relative number of lesions.

Three additional chemicals were also tested in the same way. Chlordane applied at 25, 50, and 100 p.p.m. did not affect blight development. Monuron killed the plants at 0.1 p.p.m. and so reduced leaf areas at 0.05 p.p.m. that they were not comparable to the controls. Lindane applied at 25, 50 and 100 p.p.m. also reduced leaf areas in young plants so that the number of lesions per leaf was less than that of the controls although the number per unit area was obviously greater. This was confirmed in a further experiment in which lindane and aldrin were applied at 6.2, 12.5, 25, 50 and 100 p.p.m. to roots of larger plants with fully expanded leaves. Lindane caused an increase in lesion numbers which varied with the concentration from 6.2 to 25 p.p.m., as shown in Figure 2, whereas aldrin at all concentrations brought about a marked decrease in infection.

Effect of Chemicals on Fusarium Wilt Development

In order to compare the effects on wilt development of treatments applied at different times to different lots of plants, the increase or decrease in the average wilt index of treated plants over the index of the control plants was calculated. The differences in wilt indices found following treatments with 13 chemicals at five dosage levels before and after inoculation are represented in Figure 3. The greatest increase in wilt development followed the application of lindane, particularly after inoculation.

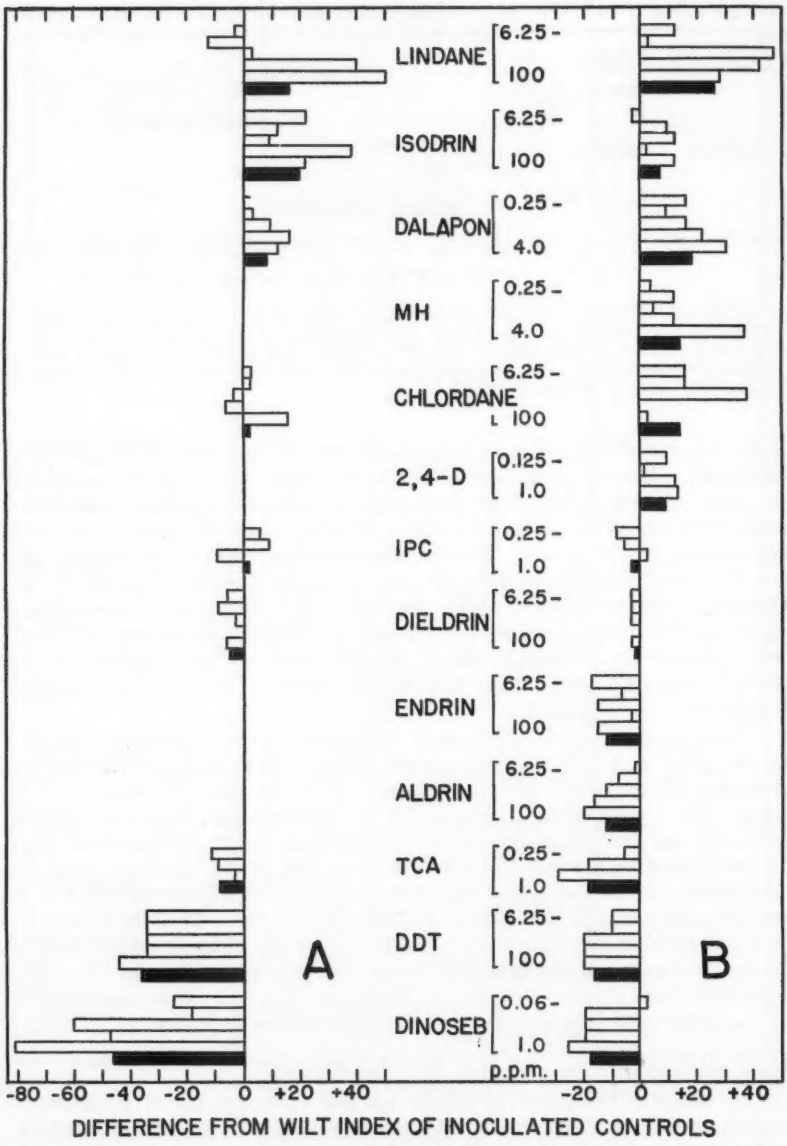

FIGURE 3. Effects of various chemicals applied to sand (A) before inoculation and (B) after inoculation of roots on development of Fusarium wilt. *White bars* from top to bottom in each group represent difference in wilt index between treated and untreated plants; *black bar* represents mean effect of all dosages in the logarithmic series.

This wilting was not all due to the pathogen, however, since the higher concentrations of lindane induced some wilting of non-inoculated plants presumably through root injury. Pre-inoculation treatments with isodrin increased wilt more than post-inoculation treatments; the reverse was true of dalapon. MH and 2,4-D applied immediately after inoculation increased wilt. Wilt development following other treatment periods with these two chemicals will be described below. Pre-inoculation treatments with chlordane were without effect and post-inoculation treatments erratic in relation to dosage rates. IPC and dieldrin had no appreciable effect at either time of application. Endrin and aldrin reduced wilt when applied after inoculation but were not tested as pre-inoculation treatments with this series. TCA applied after inoculation reduced wilt to degrees proportional to the dosage but was less effective when applied before inoculation. Pre-inoculation treatments with DDT reduced wilt more than post-inoculation treatments and the dosages were almost equally effective. Dinoseb had the greatest effect on wilt development of any of the chemicals tested, particularly as a pre-inoculation treatment.

The effect on wilt development of varying the treatment period in relation to time of inoculation was investigated further with the two growth-regulating herbicides 2,4-D and MH and three tomato varieties which differed in their susceptibility to Fusarium wilt. Young plants of the susceptible variety John Baer, the resistant variety Pritchard, and the immune variety Pan America were divided into three groups. Solutions containing 0.5 and 1.0 p.p.m. 2,4-D or 4.0 and 8.0 MH were applied daily to the sand in which they were growing, beginning 7 days prior to inoculation with one group, immediately after inoculation with the second group, and 5 days after inoculation with the third group. All applications were continued until 10 days after inoculation.

TABLE 1.—EFFECT OF 2,4-D AND MH APPLIED DAILY TO SAND OVER DIFFERENT PERIODS ON THE DEVELOPMENT OF FUSARIUM WILT IN SUSCEPTIBLE (JOHN BAER) AND RESISTANT (PRITCHARD) TOMATOES

Variety	Treatment		Time of first application					
			7 days before inoculation		Immediately after inoculation		5 days after inoculation	
			Wilted plants	Mean[1] wilt index	Wilted plants	Mean[1] wilt index	Wilted plants	Mean[1] wilt index
John Baer	Water		8	31.2	3	18.8	5	15.6
	2,4-D	0.5 p.p.m.	2	9.4	7	31.2	8	65.6
		1.0 p.p.m.	4	28.1	8	59.4	8	81.2
	MH	4.0 p.p.m.	7	34.4	6	18.8	5	18.8
		8.0 p.p.m.	7	43.8	7	43.8	5	28.1
Pritchard	Water		2	6.2	1	3.1	0	0
	2,4-D	0.5 p.p.m.	2	12.5	1	3.1	2	9.4
		1.0 p.p.m.	3	18.8	2	15.6	3	18.8
	MH	4.0 p.p.m.	8	31.2	4	12.5	0	0
		8.0 p.p.m.	7	25.0	7	31.2	0	0

[1] Average of 8 replicate plants

No evidence of infection was found in any of the Pan America plants regardless of treatment, although all inoculated plants were shorter than their corresponding control plants at the end of the experiment. No wilting or vascular browning developed, nor could the presence of mycelium in the vessel be demonstrated by plating stem sections on water agar. The 2,4-D and MH treatments, therefore, apparently did not alter the reaction of this immune variety to Fusarium wilt.

The observations made on the other two varieties are summarized in Table I which shows the number of wilted plants and the mean wilt index of the eight replicate plants receiving each treatment. Applications of 2,4-D which started 7 days before inoculation reduced wilt development in John Baer plants, whereas both later applications increased its severity, especially those started 5 days after inoculation. MH, on the other hand, increased wilt development with all three treatment periods but its effect was greatest with the pre-inoculation treatment and least with the delayed treatment. In the resistant variety Pritchard, 2,4-D increased wilt to about the same degree regardless of application time. MH increased wilt in this variety when applied before or immediately after inoculation but had no effect when applied later.

Effect of Chemicals on Fungi in Culture

The toxicity of the various insecticides and herbicides to *F. oxysporum* in culture was determined by the method used with *Helminthosporium sativum* (9). Again, dinoseb was the only chemical showing fungitoxicity, and its effect varied with the acidity of the medium. At pH 3.5 growth of *F. oxysporum* was retarded by 1.25 p.p.m. and completely inhibited by 2.5 p.p.m. of dinoseb; at pH 5.5 growth was only retarded by 10 p.p.m.; at pH 7.5 growth was not affected by 10 p.p.m.

DISCUSSION

From the results of these experiments it is evident that many of the insecticidal and herbicidal chemicals present in soil, owing to direct application or accumulation from foliage application, can potentially affect the development of plant diseases caused by either soil-borne or air-borne pathogens.

To account for the effect of such chemicals on the course of development of a plant disease, two alternative mechanisms of action may be considered: Either the chemical acts directly on the pathogen to reduce its infectivity, or it acts on the host in some way which increases or decreases its resistance to infection. The first alternative could apply only in the case of dinoseb which reduced both Fusarium wilt of tomato and barley seedling blight, caused by soil-borne organisms, but did not affect tomato early blight where the chemical did not come in direct contact with the causal organism. It was the only chemical found by culture tests to be fungicidal at the concentrations applied. Chappell and Miller (2) reported that dinoseb inhibited four other pathogenic fungi in addition to *F. oxysporum* f. *lycopersici* in culture. They also found that a reduction

of stem rot of peanuts, caused by *Sclerotium rolfsii*, and of peanut leafspot, caused by *Cercespora arachidicola*, followed pre-emergence application of this herbicide to soil. Reduction of inoculum in the soil due to the chemical was suggested as one explanation of the reduced infection. Campbell (1) reported that pre-emergence application of dinoseb reduced mint rust infection, caused by *Puccinia menthae*, by destroying the basidiospores formed from overwintered teliospores in the spring at the soil surface.

Modification of disease resistance through alteration of the metabolism of the host by growth-regulating herbicides has been investigated and discussed by several workers, (3, 4, 6, 8, 10, 12, 13). The effects on early blight and Fusarium wilt induced by chemicals of this type as they were applied in the present study are similar to the effects reported to result from their application to tomato foliage. The author found that 2,4-D applied to sand cultures over a 10-day period before inoculation of the foliage with *A. solani* increased early blight infection as did Rowell (10) when he applied it to soil 2 days before inoculation or to foliage 2 days after. The present study showed that applications of 2,4-D to sand starting a week before inoculation reduced Fusarium wilt, whereas applications started 0 and 5 days after inoculation progressively increased its severity. When Davis and Dimond (3) applied 2,4-D to tomato foliage 10 days before inoculation, no symptoms developed; treatment 4 days before inoculation reduced wilt, while treatment 4 days after inoculation had little effect. The author found that MH treatments applied to sand increased Fusarium wilt when started before inoculation, were less effective when started immediately after inoculation, and ineffective when started later. These results appear contrary to those obtained by Waggoner and Dimond (13) from foliage applications. They found that treatment at the time of inoculation caused the greatest increase in wilt, and the effect of previous treatments declined with the length of time between treatment and inoculation. The difference in results may be due to the fact that single applications were made to the foliage whereas the daily applications to sand may have had a cumulative effect.

Alteration of host metabolism is undoubtedly a factor which accounts for differences in disease development brought about by chemicals other than the growth-regulators, but little information is available. It may be significant that aldrin, shown previously to reduce barley seedling blight (9), also reduced both early blight and Fusarium wilt of tomato. The reduction of early blight by demeton is also of interest since this insecticide has been shown to be effective in the control of *Cercospora* leafspot of sugarbeets (14) and powdery mildew of roses (7a) when applied as foliage spray.

Although root injury may have been a factor influencing disease development as demonstrated by Keyworth and Dimond (7), it was not possible to establish such a relationship in the present study. The most severe root injury occurred with lindane but it was not possible to separate the wilting due to infection from that caused by the injury. Premature ageing of foliage induced by root injury could account for the increased development of early blight following lindane treatments.

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KERNEL MOISTURE AND YIELD OF CORN AS INFLUENCED BY PRE-HARVEST FOLIAR DESICCATION[1]

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[Received for publication June 17, 1958]

ABSTRACT

Experiments conducted in 1956 and 1957 showed that pre-harvest chemical desiccation of the foliage did not accelerate appreciably the loss of moisture from corn kernels. Severing of the stalks 6 inches above ground had no effect on the drying rate of the grain. Both treatments reduced yields substantially.

INTRODUCTION

Defoliants and desiccant pre-harvest sprays have been used successfully to bring about more rapid drying of the seed and as an aid to mechanical harvesting in such crops as cotton (1), alfalfa for seed (5), and field beans (7). This has stimulated interest in their use on corn as a possible means of accelerating the rate at which the ears lose moisture. If this could be accomplished without a substantial reduction in yield it would benefit the corn growers in the northern areas, particularly in years when adverse weather delays maturity and slows drying of the grain.

In 1952, Cooper (2) applied sodium cyanamid and endothal to corn of two maturities. The moisture reduction in treated corn was most noticeable in the late maturing single cross and was most evident 20 days after treatment. However, by the time safe storage levels of moisture had been reached only minor differences were found between the treated and the untreated plots. These experiments were conducted under unusually favourable drying conditions. Cooper suggested that a more complete kill of the stalk might be necessary before faster drying could be achieved.

Ham and Willard (4) top-killed hybrid Ohio W64 with two herbicides when the grain contained about 50 per cent moisture. At the same time half of the plots were cut and stored, one stalk thick, on moisture-proof paper. It was found that top-killing did not speed drying sufficiently to justify treatment, the treated and untreated plots differing by less than 2 per cent moisture. Similarly, the difference between corn left standing and that which was cut never exceeded 2 per cent, which indicated that any water furnished the plants by the roots at this time has little effect on the rate of ear drying.

Shafer (6) conducted preliminary screening trials with seven chemicals on a field of hybrid corn which averaged about 35 per cent moisture at time of treatment. One week later the untreated check contained approximately 33 per cent moisture and the treated plots ranged from 26.18 to 33.34 per cent moisture. As the moisture content approached 20 per cent differences between the treated and untreated corn became less. This work indicated that some chemical treatments were able to reduce the moisture content more rapidly than natural drying, especially during the 2-week period following application.

[1] Contribution from the Forage Crops Division, Experimental Farms Service, Ottawa, Ont.

Crane (3) studied the effect of pre-harvest spraying with endothal and of hand defoliation on the rate of ear drying in three corn hybrids with inherently different rates of drying. He found that the spray did not increase significantly the rate of moisture loss and hand defoliation only gave a small but significant difference in one of the two years. No interaction was found between the hybrids and the treatments used. A treatment is also reported for one year in which ears were detached from the stalk with shanks and husks intact. The detached ears dried significantly faster than those in the other treatments.

The effect of the pre-harvest desiccation of the foliage on the grain yield of corn has not been reported by the above workers.

MATERIALS AND METHODS

To study the effect of pre-harvest chemical dessication of the foliage and of cutting the plants on the rate of ear drying and yield of field corn, experiments were conducted in 1956 and 1957 with the following four treatments: untreated, stalks left standing (check); sprayed with a desiccant, stalks left standing; untreated, stalks cut and stood on waterproof paper; sprayed with a desiccant, stalks cut and stood on waterproof paper. The latter two treatments were included to determine if any water that might be taken up by the plant after treatment had an influence on the rate of ear drying.

The four treatments were replicated four times in a Latin square design. Plot size was six 50-foot rows, two of the four centre rows being designated for moisture determinations and two rows for yield. Two kernels were planted every 20 inches in rows spaced at 40 inches. The single cross hybrid WF9 × M14 was used. The tests were sprayed with DDT to keep corn borer damage to a minimum. Moisture determinations were made by the oven method. A 12 per cent solution of liquid cyanamid was used as the desiccant applied at the rate of 50 gallons per acre which, from past experience, was known to give a thorough and rapid kill of the foliage. In the plots designated for cutting the stalks were severed 6 inches above the ground and stood in small shocks on waterproof paper in a manner that would permit the free movement of air through the shocks. The treatments were carried out when the kernel moisture was approximately 50 per cent. Subsequent moisture determinations were made at weekly intervals until harvest but for simplication of the tables are not all reported.

RESULTS AND DISCUSSION

The results of the 1956 experiment are given in Table 1. The corn which was sprayed with the desiccant and left standing dried faster during the second week after treatment than the untreated and maintained this differential until harvest, October 23. Although these differences in kernel moisture were statistically significant they never exceeded 3.3 per cent, which shows that the pre-harvest treatment did not hasten the drying of the grain appreciably. Whether the frost, which occurred 9 days after treatment, had masked the effect of the treatment could not be determined.

TABLE 1.—EFFECT OF PRE-HARVEST TREATMENTS ON KERNEL MOISTURE AND YIELD OF CORN, 1956

Treatment	Kernel moisture, per cent					Yield, shelled corn	Yield reduction
	Sept. 11	Sept. 18	Sept. 25	Oct. 9	Oct. 23		
						bu./ac.	%
Stalks left standing							
Untreated	51.2	42.2	38.8	29.8	23.6	125.9	—
Treated*	—	42.6	36.3	26.5	20.6	108.5	13.8
Stalks cut and stood on waterproof paper, Sept. 11							
Untreated	—	42.7	36.7	27.8	23.1	108.4	13.9
Treated*	—	42.3	36.0	28.3	24.1	104.5	17.0
L.S.D. (0.05)	—	N.S.	1.6	2.2	1.6	7.4	

* Foliage sprayed with liquid cyanamid solution Sept. 11. *Note:* Killing frost on Sept. 20.

TABLE 2.—EFFECT OF PRE-HARVEST TREATMENTS ON KERNEL MOISTURE AND YIELD OF CORN, 1957

Treatment	Kernel moisture, per cent					Yield, shelled corn	Yield reduction
	Sept. 17	Sept. 24	Oct. 1	Oct. 15	Oct. 29		
						bu./ac.	%
Stalks left standing							
Untreated	47.2	39.4	34.6	27.5	24.9	123.4	—
Treated*	—	40.9	35.5	28.5	24.5	111.0	10.0
Stalks cut and stood on waterproof paper, Sept. 17							
Untreated	—	39.1	34.0	27.3	24.6	108.9	11.8
Treated*	—	39.5	34.9	28.9	25.8	108.2	12.3
L.S.D. (0.05)	—	1.1	N.S.	N.S.	0.9	7.5	

* Foliage sprayed with liquid cyanamid solution Sept. 17.

On only one date was the moisture lower in the shocked corn than in the untreated, standing plots, and this occurred 2 weeks after treatment when the moisture was still above 35 per cent.

Both desiccation of the foliage and severing of the stalks reduced the yields significantly and by approximately the same amount. This loss averaged 14.9 per cent.

Data from the 1957 experiment are presented in Table 2. In this year the ears in all treatments dried at the same rate with two exceptions. Slight differences occurred on September 24 and October 29, but in neither case was chemical leaf killing favoured. Since desiccating the foliage did

not accelerate ear drying in 1957, a year in which faster drying would have been beneficial because of adverse weather conditions in the fall, this practice appears to have little value in corn.

As in the previous year, yields were reduced significantly by desiccation of the foliage and by severing of the stalks, the loss averaging 11.4 per cent. This work indicates that, if the foliage of corn is desiccated or the stalks severed when kernel moisture is approximately 50 per cent, a reduction of at least 10 per cent in yield can be expected.

The results of these two experiments show that severing the stalks does not speed up drying of the ears. Therefore, if the roots continue to furnish water to the corn plant when kernel moisture is below 50 per cent, this water has no influence on the drying rate of the ears. Crane (3) found that detached ears dried faster than ears left on the stalk, which he believes supports Cooper's (2) suggestion that if corn were sprayed with a chemical at a dosage which would kill the stalk and stop water movement, a greater increase in drying rate could be expected. If Cooper referred to the movement of water from the roots to the stalks the present findings would not support his suggestion, since ears on the untreated standing corn dried at the same rate as those on severed stalks. However, it seems probable that water already in the stalk at cutting time retards drying of the ears since Crane found that detached ears dried faster than ears left on the stalk.

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BROADCAST TREATMENTS FOR CONTROL OF THE CABBAGE MAGGOT IN RADISH, AND RESULTANT RESIDUES[1]

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[Received for publication July 4, 1958]

ABSTRACT

In sandy loam soil at Ottawa, heptachlor and aldrin granules, broadcast at 3 lb. of toxicant per acre before planting, gave respectively 70 to 80 and 58 to 84 per cent control of a very severe infestation of the cabbage maggot in radish planted 0, 7, 15, and 21 days after treatment. Chlordane granules at 5 lb. gave 4 to 48 per cent control. The granulated insecticides were applied to the surface of the soil with a hand fertilizer spreader and raked into the top 2 inches. Insecticide residues on the radish roots at harvest were below the tolerances recently established in Canada for this crop.

INTRODUCTION

The cabbage maggot, *Hylemya brassicae* (Bouché), is a serious pest of cruciferous crops in Ontario. Adequate control of *H. brassicae* attacking the stem crucifers such as cabbage, cauliflower, Brussels sprouts, and broccoli is obtained with certain of the chlorinated hydrocarbon insecticides. These insecticides also give good control of the maggot in turnips and rutabagas in all but muck soils (6, 10, 11, 15). However, insecticidal control of the maggot in radish has been mediocre. Experimental work on control of the maggot in radish appears to have been somewhat neglected. Although radish has been frequently used as a test crop in studies on control of the cabbage maggot (2, 3, 8, 14), most of the experiments were not designed to develop practical recommendations for protection of this crop and no data were given on insecticide residues. This is a report on the effectiveness of broadcast treatments of granular aldrin, heptachlor, and chlordane for control of the cabbage maggot in radish and on the insecticide residues resulting from their use.

MATERIALS AND METHODS

Plot Arrangement and Planting

The experimental plots were in a field of sandy loam at Merivale, Ontario, 5 miles south of the Central Experimental Farm, Ottawa. The radish, variety Comet, was seeded by hand in rows 18 inches apart, each plot consisting of a 15-foot row. The experimental design was a split-plot latin square. The plots were arranged in a $4' \times 4'$ latin square and split according to four weekly planting dates, May 6, 13, 21, and 27, assigned at random.

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TABLE 1.—CONTROL OF THE CABBAGE MAGGOT IN RADISH WITH VARIOUS GRANULAR INSECTICIDES, FOR FOUR DATES OF SEEDING, OTTAWA, ONT., 1957

Granular insecticide[1]	Toxicant per acre, lb.	Radish roots		Percentage control
		Total examined	Percentage unmarketable	
Seeded on May 6, harvested on June 11				
Heptachlor 2½%	3	655	22.7	75
Aldrin 5%	3	762	31.2	66
Chlordane 5%	5	514	47.4	48
Untreated		591	91.7	—
Seeded on May 13, harvested on June 17				
Heptachlor 2½%	3	641	23.8	70
Aldrin 5%	3	704	33.1	58
Chlordane 5%	5	504	60.3	24
Untreated		497	78.8	—
Seeded on May 21, harvested on June 24				
Heptachlor 2½%	3	723	23.0	72
Aldrin 5%	3	733	27.1	66
Chlordane 5%	5	617	77.2	4
Untreated		664	80.7	—
Seeded on May 27, harvested on July 2				
Aldrin 5%	3	541	10.2	84
Heptachlor 2½%	3	486	12.7	80
Chlordane 5%	5	394	48.5	23
Untreated		335	62.7	—
Difference required for significance at 5% level			3.9	
at 1% level			8.9	

[1] *Heptachlor*, 1 (or 3a), 4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, obtained from Green Cross Products Division, Sherwin-Williams Co. of Canada Ltd., Montreal, Que.; *aldrin*, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene, obtained from Shell Oil Co. of Canada, Toronto, Ont.; *chlordane*, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan, obtained from Green Cross Products Division, Sherwin-Williams Co. of Canada Ltd., Montreal, Que.

Insecticides

Table 1 shows the insecticides and the rates of application. The insecticides were those recommended in 1957 by the Ontario and Canada Departments of Agriculture (13) for control of the cabbage maggot attacking cabbage. The granules were mixed to a consistent volume with fine sand; the mixture was applied to the surface of the soil with a hand fertilizer spreader on May 6 and immediately incorporated into the top 2 inches of soil with a hand rake. The insecticides were formulated on attapulgite, 30-60 mesh.

Criteria of Effectiveness

Each planting was harvested at the marketable stage, roughly 5 weeks after the date of seeding. The crop from the entire plot, except the final foot of row at each end, was pulled. All of the bulbous roots, i.e., roots of marketable size, were bunched, washed in the field according to commercial

practice, and graded for maggot injury. The spindly roots were not graded because previous work at Ottawa (8) had shown that the degree of injury in these roots was the same as in the bulbous roots. The washing involved immersing the bunches in a tub of cold water for about 20 minutes and rinsing each bunch by a slight back-and-forth movement of the hand before removing it. The roots were graded as follows: *marketable*, up to one-twentieth of surface area of root with superficial scarring; *unmarketable*, deep scarring, or more than one-twentieth of surface area of root with superficial scarring, or plants killed.

Population Records

Outbreak populations of the cabbage maggot occurred in the Ottawa area in 1957. Daily counts of the eggs laid on 25 plants of early cabbage in a permanent study field at the Central Experimental Farm showed that oviposition by the insect was higher in the spring of 1957 than in any other year since egg counts were begun in 1946 and that egg-laying extended over a longer period than usual. Records for the 42-day period from May 5 to June 15 compared with the previous 10-year average were as follows:

	Total number counted	Average number per plant per day	Date first eggs found	Peak of egg-laying
1946-1955	4,130	3.9	May 12	May 26
1957	15,390	14.7	May 6	May 30

In the untreated check plots at Merivale, a large number of the radish plants, many of them almost mature, wilted and died from the damage. Weekly collections of maggots from infested roots showed that, of 209 maggots collected, 73 per cent were of *H. brassicae*. The remainder were of *H. cilicrura* (Rond.) or *H. trichodactyla* (Rond.).

Analysing Residue

Only the first planting was analysed for insecticide residues. After grading, the plants were topped and the roots sealed in polyethylene bags, which were stored overnight in a refrigerator. On the following morning they were shipped by air express to the Entomology Laboratory, Chatham, Ont. The roots were extracted on the day after they were received. External residues were extracted by tumbling the whole roots with redistilled *n*-hexane in glass jars. To determine possible internal residues the rinsed roots were ground in a Waring blender and the product was tumbled with redistilled *n*-hexane in glass jars. The extracts from aldrin- and heptachlor-treated roots were chromatographed through 10 grams of Florisil[1], 60-100 mesh, and those from the chlordane-treated roots through 5 grams of a mixture compounded from 10 parts of sodium sulphate, 5 parts of Attaclay[2], 5 parts of Hyflo Super-Cel[3], 2 parts of Nuchar[4]. Aldrin was analysed by the method of O'Donnell *et al.* (12), heptachlor by that of Jorgensen (9), and chlordane by that of Davidow (1).

[1] A synthetic analytical adsorbant (Floridin Co., Tallahassee, Florida).
[2] Attapulgite (Minerals and Chemicals Corp. of America, Menlo Park, New Jersey).
[3] Diatomite (Johns-Manville, New York, N.Y.).
[4] A form of activated carbon (Fisher Scientific Co. Ltd., Toronto, Ont.).

RESULTS

Control

Table 1 shows that heptachlor gave good control of the maggot in all four plantings. Aldrin gave moderate control in the first three plantings and good control in the last. Chlordane gave unsatisfactory control in all plantings. Heptachlor and aldrin, especially the latter, gave better protection of the roots in the final planting than in the first three, apparently because the infestation was lower in the final planting.

Residues

The insecticide residues on or in the radish roots, in p.p.m., averaged as follows:

	External rinse of whole root	Extraction of macerated root
Aldrin	0.04	>0.04
Heptachlor	>0.1	>0.1
Chlordane	>0.3	>0.3

Residues on all samples were below the official tolerances recently established in Canada for radishes, namely, 0.25 p.p.m. for aldrin, 0.3 p.p.m. for chlordane, and 0.1 p.p.m. for heptachlor.

DISCUSSION

Davis *et al.* (2) found that broadcast soil treatments of aldrin at 4 lb. per acre were less effective than those of heptachlor against the cabbage maggot in radish. At first examination, the present data appear to support those of the latter authors. However, Table 2 shows that the aldrin-treated plants produced more and bigger roots than the heptachlor-treated plants; at harvest the total weights of marketable roots were about the same. Comparison of the number of plants established indicates that the aldrin was slightly phytotoxic.

The data suggest that there may have been a moderately rapid loss in the effectiveness of chlordane. According to Fleming and Maines (5) the persistence of this insecticide is related to the organic matter content of the soil; in tests with 51 mineral soils from the Atlantic States, the more organic matter present the greater was the rate of loss of insecticidal activity, espe-

TABLE 2.—YIELDS AND STANDS OF RADISH IN HEPTACHLOR- AND ALDRIN-TREATED PLOTS, OTTAWA, ONT., 1957

Insecticide	Total number of roots, bulbous and spindly	Bulbous roots			
		Total	Average weight, gm.	Total number marketable	Total weight marketable
Heptachlor	5,049	2,505	5.49	2,007	11,018
Aldrin	4,573	2,740	5.96	2,011	11,986

cially with soils of more than 5 per cent organic matter. Forbes and King (7) and Edwards *et al.* (4) have suggested that the loss of insecticidal activity in soils of relatively high organic content is not due to degradation of the insecticide but to retention of the insecticide in a non-available state by adsorption. In the present experiment the soil had received annual applications of barnyard manure for several years and its organic matter content was determined by R. L. Halstead, Soil Chemistry Unit, Chemistry Division, Ottawa, as 7 per cent.

ACKNOWLEDGEMENT

The writers gratefully acknowledge the considerable help of L. M. Cass, who assisted in taking records and applying the insecticides.

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INFLUENCE OF GIBBERELLIC ACID ON THE NICOTINE CONTENT OF CIGAR TOBACCO[1]

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[Received for publication June 13, 1958]

ABSTRACT

Treatment of tobacco with gibberellic acid in field and greenhouse experiments had little effect on yield of leaves but decreased the nicotine content of both leaves and roots. It is postulated that the decrease of nicotine content in the tobacco leaves resulted from a change in the metabolism of roots caused by gibberellic acid applications.

INTRODUCTION

Although the effect of gibberellic acid on elongation of plants is well recognized (1, 4, 5, 10), little information is available concerning its effect on the chemical composition of plants (8). In the case of tobacco, Yabuta *et al.* (11) reported a decreased nicotine content in treated seedlings as well as a decrease in the average weight per plant. Relationships between growth responses and rate and time of application of gibberellic acid have been established (2) for some plants but not for tobacco and particularly the nicotine content of this plant. The purpose of the present work was to investigate under greenhouse and field conditions the influence of this material on the nicotine content of mature or near-mature cigar tobacco with reference to such factors as rate of application, time of application and time of harvest. In addition, information was sought on the effect of gibberellic acid on the nicotine-producing capacity of tobacco roots.

MATERIALS AND METHODS

Using cigar tobacco, *Nicotiana tabacum. L.* variety Resistant Havana 211, four greenhouse experiments, numbered 1, 2, 3, and 4, and one field experiment, were conducted. In all cases, the gibberellic acid was applied as an aqueous foliar spray. The greenhouse trials were conducted during spring and summer when generally good sunlight prevailed and temperatures were moderate. For Experiments 1, 2, and 3, details of which are given in Table 1, the tobacco was seeded in quartz sand and later transplanted to soil in 2-gallon glazed clay crocks. There were six single-plant replicates. In Experiments 1 and 3, only one set of six plants was taken to represent the check; in Experiment 2, there were three such checks.

In Experiment 3, the influence of rate and time of application of gibberellic acid on the nicotine content was investigated by harvesting one-half of the plants about 2 weeks later than the first half. In Experiment 4, which consisted of five two-plant replicates, information was sought on the nicotine content of tobacco roots. The plants were grown in sand supplied with nutrient solution (6), sprayed once with gibberellic

[1] Contribution from Tobacco Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

TABLE 1.—RATES AND DATES OF APPLICATION OF GIBBERELLIC ACID AND DATES OF TOPPING AND HARVESTING OF CIGAR TOBACCO

Experiment no.	Rate of application	Date of application			Date of topping	Date of harvest
	mg. per plant	Early	Medium	Late		
1	0.0, 0.5, 1.0, 2.0	May 14	May 23	June 10	June 10	June 21
2	0.0, 1.0, 2.0	Aug. 1	Aug. 12	Aug. 20	Aug. 21	Sept. 3
3	0.0, 0.5, 1.0, 2.0	Aug. 1	Aug. 12	Aug. 20	Aug. 21 (1) (2)	Sept. 3, 4 " 17, 18

acid solution (3.0 mg. per plant) and harvested 3 weeks later. The fine roots were washed free of sand and cut off from the main root. The leaves from greenhouse experiments were not cured.

In the field experiment there were four replications, each consisting of a two-row plot. Standard planting, cultivation, harvesting and curing practices were followed. The gibberellic acid was applied 6 days before topping at rates of 0.0, 0.5, 1.0 and 2.0 mg. per plant. Plants of greenhouse Experiments 1, 2, 3, and of field experiments were topped at the early flowering stage. Topping usually increases the nicotine content of leaves but, because of the uniform treatment, this factor did not have a bearing on the results of this investigation.

In all experiments the leaves and, where applicable, the roots were oven-dried at 80°C. and weighed. The leaf and root samples were ground in a Wiley mill and analysed for nicotine by the methods of Cundiff and Markunas (3).

The influence of gibberellic acid on nicotine production in excised roots of *Nicotiana rustica* was tested, using techniques and nutrient solution developed by Macdowall*. The roots were grown aseptically in nutrient solution with additions of gibberellic acid, and the nicotine content of the whole system was determined spectrophotometrically (9).

RESULTS AND DISCUSSION

The results of greenhouse Experiments 1, 2, and 3 are presented in Table 2.

In Experiment 1, the differences in nicotine content and leaf yield were not significant. However, values for plants treated with gibberellic acid were lower than for untreated plants. The lower nicotine content, 1.64 per cent for May 23rd treatment at 0.5 mg. per plant, was 53 per cent of that where no treatment was applied.

In Experiment 2 the differences in nicotine content between all early and medium treatments and checks were statistically significant while those of the late treatments and check were statistically not significant. The differences in dry weight of leaves were not significant.

The important aspects of the statistical analysis of Experiment 3 are shown in Table 3.

* Macdowall, F.D.H. Tobacco Division, Central Experimental Farm, Ottawa. *Unpublished data.*

TABLE 2.—INFLUENCE OF GIBBERELLIC ACID ON NICOTINE CONTENT AND YIELD OF GREENHOUSE GROWN CIGAR TOBACCO

Date of application	Date of harvest	Gibberellic acid, mg. per plant							
		0.0	0.5	1.0	2.0	0.0	0.5	1.0	2.0
		Nicotine (per cent)				Weight per plant (grams)			
Experiment 1									
		3.06				32.51			
Early, May 14	—		2.26	2.13	2.12		30.38	26.40	26.98
Medium, May 23	—		1.64	1.70	1.82		31.55	31.54	28.51
Late, June 10	—		2.11	1.88	1.95		27.89	28.05	28.61
		L.S.D. 5% N.S. (all means)							
Experiment 2									
Early, Aug. 1	—	1.94	—	1.41	1.31	30.40	—	29.40	28.30
Medium, Aug. 12	—	2.08	—	1.32	1.21	28.60	—	29.50	30.00
Late, Aug. 21	—	1.77	—	1.54	1.42	28.90	—	29.00	31.10
		L.S.D. 5% 0.41 (nicotine means)							
Experiment 3									
		3.06				32.50			
Early, Aug. 1			2.26	2.12	2.40		30.38	26.43	26.95
Medium, Aug. 12	Sept, 3, 4		1.81	1.70	1.82		31.51	31.53	28.48
Late, Aug. 21			2.11	1.88	1.95		28.88	28.03	28.60
		3.10				41.26			
Early, Aug. 1			2.93	2.87	2.40		37.40	35.51	37.35
Medium, Aug. 12	Sept. 17, 18		2.83	2.65	2.48		41.56	42.38	42.40
Late, Aug. 21			2.64	2.67	2.29		38.78	44.05	40.41

TABLE 3.—SIGNIFICANT ASPECTS OF ANALYSIS OF VARIANCE OF EXPERIMENT 3

A. Nicotine

Source	Sum. of squares	Degrees of freedom	Mean square
Including checks			
Treatments (rates)	8.48	9	0.94**
Harvest dates	9.60	1	9.60**
Harvest dates × treatments	3.29	9	0.36*
Excluding checks			
Treatments (rates)	0.65	2	0.32
Application dates	1.47	2	0.75*
Harvest dates	10.53	1	10.53**
Treatments × application dates	0.09	2	0.04
Treatments × harvest dates	1.19	2	0.59*
Application dates × harvest date	0.92	1	0.92*
Treatments × application dates × harvest dates	0.23	2	0.11
B. Weight			
Including checks			
Treatments (rates)	455.14	9	50.57
Harvest dates	354.42	1	354.42**
Harvest dates × treatments	187.10	9	20.78

*Significant at the 5% level
**Significant at the 1% level

When the values for the untreated plants were included in the statistical analysis the differences in nicotine content were significant at the 1 per cent level. Tobacco harvested at two different times showed variations in the amount of nicotine significant at the 1 per cent level; the influence of gibberellic acid was less pronounced at the late harvest date than at the early harvest. The effect of various rates of application of gibberellic acid was less pronounced at the late harvest date than at the early harvest. The various rates of gibberellic acid applied reacted similarly at the two times of harvest, as indicated by the significance of harvest date and treatment (rate) interactions.

When the values for the untreated plants were excluded, the differences in nicotine content between the various treatments were not significant, showing that the important variations were between the checks and treatments only. Differences caused by various times of application were significant, indicating that there was a most effective time of application. At the early harvest this was the medium-late application, whereas at the late harvest the late application was the most effective in reducing the nicotine content. Rate and harvest date interaction was significant: the differences in nicotine content between the various application rates were small, while those between the harvest dates were quite large. Interaction between application times and harvest dates was significant: application times did not have as much effect on the nicotine content as the harvest dates.

Statistical analysis of the leaf yield data showed that the differences between treatments were not significant, while those between the harvest dates were highly significant: at the late harvest the yield was higher than at the early harvest.

The treatment-growth response relationships may be expressed by calculating the "response index" (2). This was calculated for the average nicotine content of tobacco from Experiment 3 for each treatment, harvest date, and application time (Figure 1). Large "response index" values and points high on the curves indicate greater relative differences between the treated and untreated plants than the small values and points low on the curves.

The index curves for all treatments harvested early were similar: the highest point was reached at the medium-late application time. The 1.0 mg. treatment gave the highest response throughout. At the late harvest the responses from the 2.0 mg. treatment were higher than those

TABLE 4.—NICOTINE CONTENT, WEIGHT, AND GRADE INDEX OF FIELD-GROWN, CURED CIGAR TOBACCO TREATED WITH GIBBERELLIC ACID

	Gibberellic acid mg. per plant				L.S.D.	
	0.0	0.5	1.0	2.0	5%	1%
Nicotine (per cent)	4.17	3.40	3.35	3.57	0.28	0.40
Weight per plant (grams)	100.52	104.44	102.48	92.28	N.S.	N.S.
Grade index	21.70	20.93	22.48	22.36	N.S.	N.S.

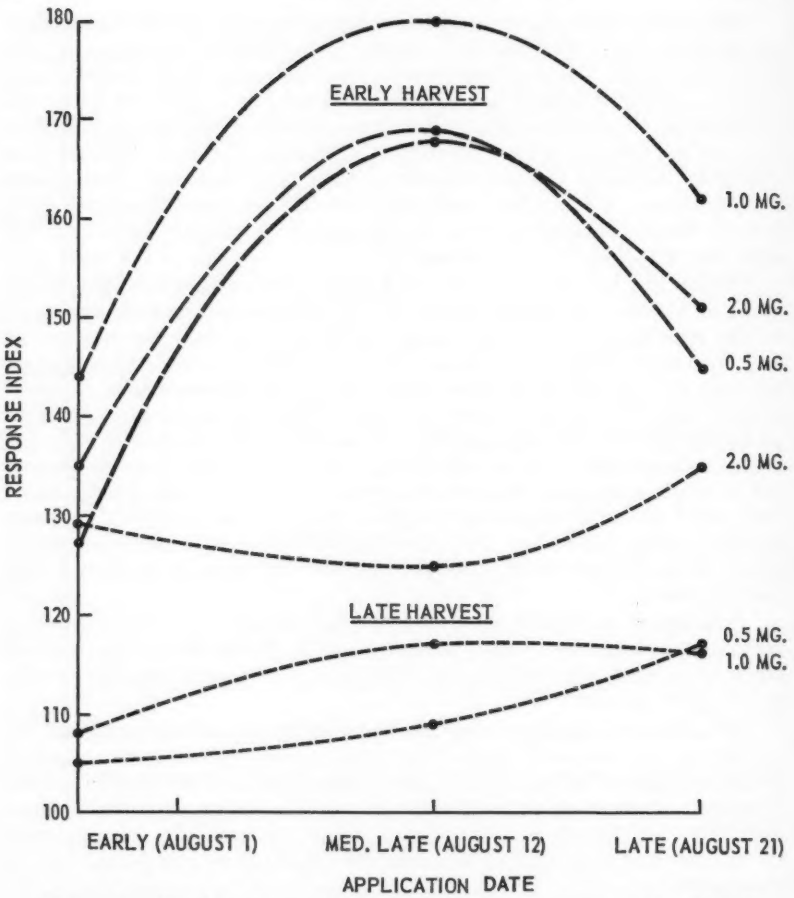

FIGURE 1. Nicotine content of tobacco related to treatment with gibberellic acid. (The shapes of connecting lines have no meaning and are used merely to connect the various points of response indices).

from the lesser treatments, indicating a carry-over influence of the large dose for a comparatively long period of time. The early and medium treatments produced less response than the late ones, revealing the reduction of the influence of the chemical. Generally, the responses were smaller and more erratic at the late than at the early harvest. This index showed that the nicotine content of tobacco may follow the plant growth responses.

In the field experiment (Table 4) the differences in nicotine content between the gibberellic acid treatments were not statistically significant, while those between the treatments and the check were highly significant. The differences in the average weight of leaves as well as of the grade index were not significant.

Experimental evidence indicates (7) that nicotine is synthesized to a very large extent in roots, particularly in the root tips, and is translocated to the stems and leaves. A question may be asked as to whether gibberellic acid hinders the translocation or the synthesis of nicotine.

Results from Experiment 4 (Table 5), where the fine roots of tobacco plants were analysed for nicotine, indicated that the roots from the treated plants contained significantly less nicotine than those from untreated ones. In view of the experimental evidence now known, it is probable that this decrease in nicotine content would have been greater had the plants been harvested less than 3 weeks after treatment.

When excised tobacco roots were grown aseptically in nutrient solutions containing various amounts of gibberellic acid, (Table 6) there was a great decrease in nicotine content in the whole system (roots and nutrient solution analysed together). The differences were numerically large and statistically significant. At the highest concentration (10^{-4}M) of gibberellic acid in the solution, the average content of nicotine was less than one-thirteenth that of the check treatments. With the same gibberellic acid treatment, the average dry weight of the roots was decreased only slightly and not significantly, while the weights in other treatments were significantly higher than those of the checks. Culture of excised tobacco roots of different species may prove to be the most rewarding approach to study the tobacco alkaloid synthesis or destruction in the presence or absence of gibberellic acid.

It appears that the translocated gibberellic acid (8) or its active metabolic products influenced the nicotine synthesizing mechanisms of tobacco roots of intact plants as well as those of excised roots. It may be further deduced that this decrease of nicotine synthesizing activity and not any possible translocation block was responsible for the decrease

TABLE 5.—NICOTINE CONTENT OF INTACT ROOTS OF CIGAR TOBACCO TREATED WITH GIBBERELLIC ACID

	Gibberellic acid mg. per plant			Necessary "F" 5%
	0.0	3.0	"F" 5%	
Nicotine (per cent)	0.435	0.356	7.52	5.12

TABLE 6.—NICOTINE CONTENT AND WEIGHT OF DETACHED ROOTS OF *Nicotiana rustica* GROWN IN NUTRIENT SOLUTIONS SUPPLIED WITH GIBBERELLIC ACID

	Concentration of gibberellic acid solution				L.S.D. 5%
	0	10^{-6}M	10^{-5}M	10^{-4}M	
Nicotine in roots and solution (per cent)	4.32	0.62	0.52	0.32	1.0048
Average weight of roots (grams)	0.0369	0.0704	0.0666	0.0326	0.00563

of nicotine content in gibberellic acid-treated tobacco leaves. A possibility may not be excluded that gibberellic acid caused destruction of nicotine and that this factor, and not the changes in the synthesizing mechanism, were responsible for the decrease of nicotine in treated tobacco leaves and roots. This approach remains to be investigated.

The time of application, the concentration of the chemical in the roots and the root-growth activity play a role in determining the final nicotine content of the leaves. The early applications may soon lose their effect due to dilution or inactivation of the active substance. The medium applications may be the most effective because of relatively large amounts of gibberellic acid in the plants, compared to early applications. At the medium time, roots are still growing and elongating actively and the chemical may exert its influence on the synthesizing mechanism. At the late application time, when the plants are flowering, the root growth, nicotine synthesis and its subsequent translocation may be naturally decreased. The different amounts of gibberellic acid used did not produce significant differences in the nicotine content of tobacco; often the 1.0 mg. treatment appeared to be more effective than higher or lower amounts.

The differences in nicotine content and weight of leaves were in no case as large in these experiments as in those reported by the Japanese investigators. In many instances, contrary to Yabuta's results, the treated plants yielded more dry matter than the untreated ones. The nicotine contents, on the other hand, were greater than in the Japanese experiments. Reasons for these divergences may be a varietal response to gibberellic acid—a flue-cured variety "Bright Yellow" was used in the experiments. The use of a relatively large amount of the chemical on small and young plants and harvesting soon after treatments may also have caused low nicotine content and low dry matter yield. There is a possibility that the 2.0 mg. treatment per seedling, used by Yabuta, was toxic. This may have been true also in the experiments reported here.

CONCLUSIONS

The conclusion may be drawn that gibberellic acid brings about a significant decrease in nicotine content not only in tobacco seedlings but also in tobacco plants approaching maturity. The decrease in nicotine content is not coupled with significant decrease or increase of yield of dry matter of leaves. The time and dose response relationships recognized for growth, and expressed by a "response index", were found applicable to the nicotine content of tobacco. It is postulated that the decrease of nicotine content in the tobacco leaves resulted from a change in the metabolism of roots, caused by gibberellic acid applications.

ACKNOWLEDGEMENTS

Thanks are due to N. A. MacRae, E. T. McEvoy and F. D. H. Macdowall for assistance with this investigation. Appreciation is expressed to the Imperial Chemical Industries, Ltd., Great Britain, and Merck and Co. Inc., Rahway, N.J., for supplies of gibberellic acid.

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CHEMICAL CONTROL OF THE NARCISSUS BULB FLY, *LAMPETIA EQUESTRIS* (F.), IN BRITISH COLUMBIA[1]

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[Received for publication June 30, 1958]

ABSTRACT

The narcissus bulb fly, *Lampetia equestris* (F.), was controlled on southern Vancouver Island with insecticides applied by four methods at planting time to narcissus bulbs grown in sandy loam soils of pH 5.9 to 6.5.

Soaking bulbs for 1.5 hours in hot-water (110° F.) and formalin mixture as for control of the bulb nematode, with heptachlor emulsifiable concentrate added at 2, 4, or 10 lb. of toxicant per 1,000 gal. gave 100 per cent control for the 2 years tested. Soaking the bulbs for 10 minutes in cold water containing emulsifiable concentrates of dieldrin or heptachlor at 3 lb. of toxicant per 100 gal., or of aldrin or chlordane at 5 lb., gave 93 to 98.5 per cent control for 3 years; DDT at 5 lb. and lindane at 0.5 lb. were not effective. A dust of dieldrin at 3 lb. of toxicant per acre, of aldrin or heptachlor at 5 lb., or of chlordane 10 lb. applied to the bulbs in the open furrow gave 95 to 99 per cent control for 3 years; lindane at 1 lb. did not give satisfactory control. A spray containing an emulsifiable concentrate of dieldrin at 1 lb. of toxicant per 100 gal. per acre, of aldrin or heptachlor at 2 lb., or of chlordane at 5 lb. gave 91 to 96 per cent control for the 2 years tested. The soil in this test had the highest organic matter content (13 per cent); the others were moderately high with 5 to 8 per cent. Lindane in the cold-water soak treatment was the only insecticide that adversely affected bulb growth.

INTRODUCTION

The narcissus bulb fly, *Lampetia equestris* (F.), is the most important insect pest of narcissus bulbs wherever they are grown (1, 6, 13). In British Columbia, where most of the bulbs are grown for the cut flower trade and remain in the soil for 3 years, the infestation was as high as 75 per cent in some fields during 1949 (1).

Bulbs are damaged by larvae feeding in them. The larvae hatch from eggs deposited on the soil surface during May and June.

Until 1953 methods and materials recommended for preventing damage were directed at killing the adults (1, 11–13), preventing egg-laying by using repellents (10, 13), and killing the eggs or the larvae before they reached the bulbs (1, 6, 8, 13, 15). These methods are not adequate and require frequent applications because the insecticide either acts too slowly or lacks sufficient residual effect. Some protection is obtained by hilling the soil in the rows and digging the bulbs each year early in July (6, 12–13).

Methods for controlling the larvae in the bulbs after harvest include the hot-water treatment (6–7, 13), methyl bromide fumigation (2, 6), and BHC soak (13). However, these after-harvest treatments are not satisfactory since the bulbs are usually not dug until about 2 months after the larvae start feeding, and considerable damage to the flower parts in the bulbs has already occurred (3).

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In 1953, Doucette (9) reported that bulbs were protected for 1 year after a pre-planting soak treatment in water containing aldrin, chlordane, or heptachlor. Experiments conducted in 1955 and 1956 by Woodville (16–17) with aldrin, dieldrin, chlordane, and heptachlor in cold- and hot-water soak treatments indicated that satisfactory control was obtained for 2 years after planting.

From 1952 to 1957 experiments were conducted in British Columbia to determine the most effective methods and materials to protect the bulbs for 3 years. This is a report on the effectiveness of four methods of applying six insecticides at planting time and their effects on bulb growth.

MATERIALS AND METHODS

1. *Cold-Water Soak Treatments*

King Alfred bulbs were submerged in water containing emulsifiable concentrates before they were planted during October, 1952. These materials were mixed with 25 gal. of water in 50-gal. wooden barrels at the rates indicated in Table 1. Thirty sacks, each containing 80 lb. of bulbs, were soaked in each mixture for 10 minutes, removed, and drained. The treated bulbs remained in the sacks not more than 2 days before they were planted in rows 3 feet apart in the field.

The planting area was 1.6 acres of level sandy loam at Cordova Bay (pH 6.3). Bulb infestations had averaged 20 per cent in this field for the previous 2 years. Seven treatments were replicated three times in randomized blocks, each plot consisting of two rows, 200 yards long. Four or five sacks of treated bulbs were planted per row with a two-row planter mounted on a tractor. The bulbs were immediately covered with 4 to 6 inches of soil by disks following the planter.

The effects of the insecticides on flower stem length and root weights were determined by forcing 24 No. 1 single-nose bulbs for each treatment. These bulbs were planted in boxes measuring 12 x 6 x 5 inches. Three replicates of eight bulbs each were grown in a greenhouse at 60 to 65° F. from October 9, 1952, to February 8, 1953, at the Experimental Farm,

TABLE 1.—AVERAGE PERCENTAGES OF KING ALFRED NARCISSUS BULBS DAMAGED BY LARVAE OF THE NARCISSUS BULB FLY FROM 1953 TO 1955 AFTER A 10-MINUTE SOAK, IN COLD WATER CONTAINING VARIOUS INSECTICIDES AT PLANTING TIME IN SEPTEMBER, 1952

Emulsifiable concentrate	Toxicant per 100 gal., lb.	Bulbs damaged, %		
		1953	1954	1955
Heptachlor, 24%	3	1.4	1.3	4.6
Aldrin, 24%	5	1.4	3.6	6.6
Dieldrin, 18%	3	1.4	4.6	4.3
Chlordane, 65%	5	0.0	5.6	5.6
Lindane, 5%	0.5	13.3	12.3	17.3
DDT, 25%	5	21.0	14.0	19.0
Untreated	—	18.1	16.3	22.0
Difference necessary for significance at 5% level	—	3.9	6.4	5.7

TABLE 2.—AVERAGE PERCENTAGES OF SIR WATKIN NARCISSUS BULB DAMAGED BY LARVAE OF THE NARCISSUS BULB FLY FROM 1953 TO 1955 AFTER INSECTICIDE DUSTS WERE APPLIED ON THEM IN THE OPEN FURROW IN SEPTEMBER, 1952

Insecticide dust	Toxicant per acre, lb.	Bulbs damaged, %		
		1953	1954	1955
Aldrin, 2.5%	5	1.0	1.0	1.0
Heptachlor, 2.5%	5	0.7	1.3	1.3
Chlordane, 5.0%	10	1.8	0.6	2.3
Dieldrin, 1.5%	3	4.8	1.6	5.0
Lindane, 0.5%	1	14.8	14.6	18.6
Untreated	—	16.0	35.6	33.6
Difference necessary for significance at 5% level	—	4.5	4.7	8.9

Canada Department of Agriculture, Saanichton, B. C. Records were kept of flower stem length and wet-root weights.

2. *Dust Treatments*

Dusts were applied to Sir Watkin narcissus bulbs in open furrows before they were covered with soil during September, 1952. This test was conducted on very light, sandy loam soil (pH 6.3). Six rows planted 3 feet apart were divided into plots 40 feet long in a 6-x-6 latin square design.

The dusts (Table 2) were applied in an 8-inch band, with an 18-inch fertilizer hand-cart adjusted to apply 200 lb. per acre. The bulbs were immediately covered with 4 to 6 inches of soil.

To determine the effects of the treatments on bulb growth, four No. 1 and four No. 2 single-nose bulbs were planted on October 9 in each of 18 forcing boxes. Before being covered with soil they were treated like the bulbs in the field plots. The amount of dust applied to eight bulbs in each box, calculated on the basis of 200 lb. per acre in an 8-inch band with 3 feet between rows and bulbs 4 inches apart in the row, was 6.3 grams. Six treatments were replicated three times. The bulbs were forced in the greenhouse and records kept as described for the cold-water soak treatments.

3. *Spray Treatments*

Sprays containing emulsifiable concentrates were applied to King Alfred bulbs in open furrows in silt loam high in organic matter (pH 6.5) during September, 1953. These bulbs were planted in rows 3 feet apart, with a tractor-mounted two-row planter. Each plot was four rows wide and 200 yards long. Five treatments were replicated three times in a randomized block design. The insecticides were mixed with water in a 50-gal. metal barrel and applied by a row-crop sprayer attached to a tractor power-take-off (4) at the rates indicated in Table 4. The spray boom had two flat-type, Tee Jet 8008 nozzles per row. The boom was attached to the front of the tractor so that each nozzle was 8 inches above the bulbs and directed at a 45-degree angle to cover a 10-inch swath. One hundred gallons of spray per acre was applied at a tractor speed of 3 m.p.h. and a pump pressure of 100 p.s.i.

TABLE 3.—AVERAGE LENGTH OF FLOWER STEMS AND AVERAGE WEIGHT OF WET ROOTS OF KING ALFRED NARCISSUS BULBS FORCED IN GREENHOUSE AFTER TWO TYPES OF TREATMENT AT PLANTING TIME, OCTOBER, 1952

Insecticide	Average length of flower stems, in.		Average weight of wet roots of 8 bulbs, gm.	
	Dust treatment	Soak treatment	Dust treatment	Soak treatment
Lindane	15.9	14.4	99.0	43.0
Aldrin	16.4	16.0	125.0	116.7
Chlordane	14.6	15.9	105.0	124.0
DDT	16.4	17.0	120.0	144.7
Dieldrin	14.7	15.6	125.3	114.0
Heptachlor	13.9	17.2	118.7	145.0
Untreated	15.6	16.0	106.7	131.3
Difference necessary for significance at 5% level	—	—	—	43.8

4. *Hot-Water-Formalin*-Insecticide Treatments*

Since heptachlor was very effective in the earlier experiments, it was tested in August, 1955, as an additive to the hot-water-formalin treatment, recommended for control of the bulb nematode, *Ditylenchus* spp. (5, 7). A cement tank containing 1,000 gal. of water maintained at 110° F. by the injection of steam was used to treat King Alfred bulbs. To agitate the mixture, an electrically operated propeller in one end of the tank was rotated continuously. Five gallons of formalin were first added to the water; then heptachlor emulsifiable concentrate was added at the rate of 1, 2, 4, and 10 lb. of toxicant per 1,000 gal. About 16,000 bulbs were placed on wooden trays and submerged in each mixture for 1.5 hours and then stored for 6 to 7 weeks before planting. For each treatment, 300 No. 2 single-nose bulbs were selected and weighed. These were planted in coarse sandy loam (pH 5.0) on October 12 in single-row plots, each containing 100 bulbs, at the Experimental Farm, Saanichton. Each row was one of three replicates in a randomized block design. An additional 24 No. 2 single-nose bulbs per treatment were planted, eight per box, for greenhouse forcing. The remainder of the treated bulbs were planted by machine, during October, in two-row plots replicated three times in randomized blocks in a grower's field of fine sandy loam (pH 5.0).

No other insecticide or fungicide was applied during the experiments. The pesticides used were as follows: Aldrin and diedrin; chlordane and heptachlor; lindane and 5 per cent DDT dust; 25 per cent DDT emulsifiable concentrate; formalin (40 per cent formaldehyde).

Sampling, and Assessing Results

Since the bulbs remained in the soil for 2 or 3 years after treatment, samples were dug each year with a hand ditch-shovel. Either 50 or 100 bulbs, depending on the size of the plots, were removed from each replicate by digging one bulb at intervals of 1 yard in the row. Buffer strips 3 yards

*40 per cent formaldehyde

long, at the ends of each plot, were not sampled. Samples were taken in late August or September, when larval damage to infested bulbs was well advanced and easily seen with the naked eye.

The percentage of bulbs with larval entries was determined by scraping the basal plate of each bulb with a pocket knife to remove the dead tissue and expose the entry hole. By the middle of August the entry hole is easily found, as the surrounding tissue has decayed and turned a rusty-brown colour. All bulbs with entry holes were recorded. However, according to Hodson (12), up to 20 per cent of the larvae that enter die before they are 1 week old. This observation was verified in the Victoria work, when at least 4,000 discarded bulbs were examined and 20 to 25 per cent of those with entry holes contained dead larvae. Therefore, in this report, the percentage of bulbs with entries denotes bulbs damaged rather than bulbs containing living larvae.

Scraped and unscraped bulbs that were not infested were grown simultaneously in the greenhouse and no differences in growth were observed. Thus, this method is a reliable and non-injurious means of determining larval entry in large numbers of bulbs in the field.

RESULTS AND DISCUSSION

Good residual control of the larvae was obtained for 2 to 3 years with each type of treatment.

The cold-water soak treatments with aldrin, dieldrin, chlordane, and heptachlor resulted in less than 1.5 per cent of bulbs damaged the first year and 4.3 to 6.6 per cent the third year, compared to 16 to 22 per cent of the untreated bulbs (Table 1). DDT and lindane were not effective. Single-nose bulbs forced in the greenhouse showed no significant differences in lengths of flower stems (Table 3). Lindane was the only insecticide that gave significantly lower wet-root weights.

The dust treatments with aldrin, heptachlor, chlordane, and dieldrin showed 1, 1.3, 2.3, and 5 per cent of bulbs damaged respectively the third year compared to 33.6 per cent the untreated bulbs (Table 2). Lindane did not give satisfactory control. Treated bulbs forced in the greenhouse did not show significant differences in lengths of flower stems or in wet-root weights (Table 3).

TABLE 4.—AVERAGE PERCENTAGES OF KING ALFRED NARCISSUS BULBS DAMAGED BY LARVAE OF THE NARCISSUS BULB FLY FROM 1954 TO 1955 AFTER VARIOUS SPRAYS WERE APPLIED ON THEM IN THE OPEN FURROW IN SEPTEMBER, 1953

Emulsifiable concentrate	Toxicant per 100 gal., lb.	Bulbs, damaged, %	
		1954	1955
Aldrin, 24%	2	2.0	6.3
Dieldrin, 18%	1	2.3	4.6
Heptachlor, 24%	2	2.7	3.6
Chlordane, 65%	5	4.0	9.0
Untreated	—	14.3	16.3
Difference necessary for significance at 5% level	—	4.6	6.2

TABLE 5.—AVERAGE WEIGHT INCREASES OF KING ALFRED NARCISSUS BULBS IN 1956 AND AVERAGE PERCENTAGES DAMAGED BY LARVAE OF THE NARCISSUS BULB FLY IN 1956 AND 1957 AFTER SOAKING FOR 1.5 HOURS IN HOT WATER (111° F.) CONTAINING FORMALIN AND VARIOUS RATES OF HEPTACHLOR BEFORE PLANTING IN AUGUST, 1955

Pounds of heptachlor per 1000 gal.	Increase per 100 bulbs, oz.	Bulbs damaged, %	
		1956	1957
0	45.0	10.0	16.0
1	58.7	1.6	2.0
2	41.7	0	0
4	46.3	0	0
10	47.7	0	0

The spray treatments with heptachlor, dieldrin, aldrin, and chlordane applied at less than one-half the rates used in the dust treatments resulted in 3.6 to 9 per cent of bulbs damaged the second year (Table 4).

Adding heptachlor at 2, 4, and 10 lb. per 1,000 gal. to the hot-water-formalin mixture for control of bulb nematode, gave 100 per cent control for the 2 years tested (Table 5). The average increases in weight per 100 bulbs did not differ significantly.

None of the insecticides used produced differences in dates of flowering in the field or in the greenhouse.

Results did not differ greatly with these four treatments, where bulbs were grown in sandy loam soils of pH 5.9 to 6.5. The hot- and cold-water and dust treatments were tested in soils with an organic matter content of 5 to 8 per cent. The high organic matter (13 per cent) of the soil where the sprayed bulbs were grown may not have been responsible for the lower percentage control, as lower dosages of insecticide were used in this treatment.

There was an indication of possible movement of the hydrocarbon insecticides in the bulb tissue (Table 1). Single-nose ("rounds") bulbs soaked in water containing these materials and planted in 1953 became "double-nose" bulbs in 1954 and developed "slabs" in 1955, which became "rounds" in 1956. These "rounds", or progeny, had less than 5 per cent damaged 3 years after the original bulbs were treated, compared with the untreated bulbs that had 22 per cent damaged.

ACKNOWLEDGEMENTS

The authors wish to thank J. Raine and N. V. Tonks, Assistant Entomologists, and B. D. Ainscough and T. L. Theaker, Assistant Technicians, at the Victoria laboratory, for their assistance in checking bulb samples. The assistance of J. Crossley, Horticulturist, and S. Arrowsmith, Greenhouseman, at the Canada Experimental Farm, Saanichton, B. C., in forcing the bulbs and recording growth data is gratefully acknowledged. The authors are indebted to J. H. Neufeld, Soil Analyst, Provincial Department of Agriculture, Victoria, for the data on soil pH and organic matter, and to the following growers, who provided land and bulbs for the experiments: W. Mattick, J. Thompson, G. Vantreight, and C. R. and Ian Wilson.

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STUDIES ON SEED DORMANCY, PLANT DEVELOPMENT, AND CHEMICAL CONTROL OF TARTARY BUCKWHEAT (*FAGOPYRUM TATARICUM* (L.) GAERTN.)

III. CHEMICAL CONTROL AND EFFECTS OF COMPETITION[1]

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[Received for publication June 19, 1958]

ABSTRACT

The effects on Tartary buckwheat and barley of various chemical treatments, applied at different stages of plant development, were compared in field plots. For control of Tartary buckwheat there was no advantage in using two applications of 2,4-D (2,4-dichlorophenoxyacetic acid, ethyl ester) or of MCPA (methylchlorophenoxyacetic acid, butyl ester), 12 days apart, rather than a single application at a correspondingly higher rate. At comparable rates a low volatile ester (butoxyethanol ester) was more effective than regular 2,4-D ester (ethyl ester), especially at the later growth stages, and both were much superior to MCPA.

In competition between Tartary buckwheat plants, total production of vegetative matter and seed increased somewhat with increasing number of plants per square yard, but, as expected, the single-plant yields decreased sharply. In barley plots infested with Tartary buckwheat sprayed with LV 2,4-D when the plants had 2–3 leaves, up to 50 buckwheat plants per square yard had not yet, during the time before spraying, affected the ultimate yield of grain. On sprayed plots there was a balancing effect between herbicidal injury to the grain and decreased weed competition. The effect of the interaction between these two factors was manifested in the yield of grain.

INTRODUCTION

Although chemical control of weeds is now a recommended and widely accepted practice there are several species for which no practical or economical method of eradication has yet been found. Weeds, chiefly through their competition with crop plants, cause tremendous losses each year (3). For example the results of work by Friesen (2) indicated that the presence of weeds in crops caused an average reduction in seed yield of 15.9 per cent, considering four crops at a total of 50 different locations.

The importance of Tartary buckwheat as an annual weed has been discussed in the authors' previous papers dealing with its seed and plant characteristics and development (7,8). There has been fairly extensive research on chemical control of this weed (4,5). Nevertheless, its relative degree of resistance to common selective herbicides has provided considerable scope for further investigations concerning rates and times of application of herbicides and their effects on different populations of buckwheat plants per unit area in the crop. Comparisons of various chemicals on standard infestations of buckwheat and the use of one of the more effective of these chemicals on different populations of the weed in barley received attention in the present study.

[1] Paper based on M.Sc. thesis of the senior author, Division of Crop Ecology, University of Alberta, Edmonton, Alta., March 1958.

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MATERIALS AND METHODS

Measured amounts of after-ripened Tartary buckwheat seed were planted approximately 1 inch deep in 10-foot rows with a V-belt seeder. Where barley was included in the field experiments, it was seeded at the same depth as the buckwheat with a Columbia seeder. The experimental design of the field experiments was of the "split plot" type in all cases where both grain and Tartary buckwheat were included in the experiment. There were four replicates.

The chemical treatments were applied at 30-35 lb. pressure, using a portable motor-driven air-compressor connected to a sprayer head which was clamped to the top of a milk-bottle containing the liquid. Each bottle contained the amount of chemical required for treatment of one plot, mixed with an amount of water equivalent to 50 gal./a.

Comparison of Herbicides, 1956

Field plots containing two rows of Olli barley with two rows of Tartary buckwheat on either side were treated with single and repeated applications of the butyl ester of methylchlorophenoxyacetic acid (MCPA), the ethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D), and the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (LV 2,4-D). The first application consisted of dosages of 0, 4, 8, and 16 oz./a. of these three chemicals, and was followed 12 days later by a second application consisting of 4 oz./a. of each chemical. All possible combinations of first and second treatments (48 in all) were included. At the time of the first application (20 days after planting) the buckwheat plants had three true leaves, and were 2 inches tall, while the barley had three to four leaves, and was 7 inches in height. When the second application was made, the buckwheat was 7 to 8 inches tall, and the barley 15 to 16 inches in height. When the Tartary buckwheat plants on the check plots were beginning to set seed (33 days after first spraying) one row of buckwheat was harvested from each plot, and the fresh weight determined immediately. On September 5 (110 days after planting) another row of buckwheat was harvested, and the seed yield was determined.

Comparison of Herbicides, 1957

Field plots containing four rows of Gateway barley alternated with three rows of buckwheat (spaced 6 inches) were treated with: 4, 8, and 12 oz./a. of the ethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D); 4, 8, and 12 oz./a. of the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (LV 2,4-D); 8, 16, and 24 oz./a. of butyl (2,4-dichlorophenoxy) butyrate (2,4-DB); 2.5, 5, and 10 lb./a. of 3-(3,4-dichlorophenyl)-1-methyl-1-n-butylurea (neburon).

When the first application was made 14 days after planting, the first true leaf of the buckwheat was just beginning to unfold; the barley had two leaves, and was 4 to 5 inches tall. Ten days later, when the buckwheat had four true leaves, and the barley was 7 inches tall, with five leaves, another application was made on plots not treated previously. Two rows of barley and one of Tartary buckwheat were harvested for yield determinations. The number of buckwheat plants in the row harvested was determined both prior to spraying and at harvest time.

TABLE 1.—FRESH WEIGHT AND SEED YIELD OF TARTARY BUCKWHEAT FROM PLOTS GIVEN ONE APPLICATION OR TWO (12 DAYS APART) OF MCPA, 2,4-D, AND LV 2,4-D IN 1956

Treatment (1)		Fresh weight in lb./a.				Seed yield in lb./a.			
		One treatment (1)	Two treatments (1) + 4 oz./a. of:			One treatment (1)	Two treatments (1) + 4 oz./a. of:		
Chemical	Rate	—	MCPA	2,4-D	LV 2,4-D	—	MCPA	2,4-D	LV 2,4-D
Check	—	29,770	26,529	24,248	20,407	4,995	3,973	3,145	2,437
MCPA	4 oz./a.	18,246	12,844	12,604	11,404	3,517	2,425	1,777	1,885
2,4-D	4 oz./a.	13,685	9,483	8,763	6,002	3,121	1,777	1,645	1,356
LV 2,4-D	4 oz./a.	13,204	6,122	9,483	6,962	2,761	1,236	1,080	984
MCPA	8 oz./a.	12,844	7,923	6,482	4,561	2,281	1,681	1,500	1,140
2,4-D	8 oz./a.	3,961	1,560	2,521	1,440	1,224	564	564	456
LV 2,4-D	8 oz./a.	1,681	600	1,200	840	1,116	156	204	60
MCPA	16 oz./a.	840	720	480	360	1,140	360	120	360
2,4-D	16 oz./a.	840	240	240	240	396	204	204	204
LV 2,4-D	16 oz./a.	40	0	0	0	120	228	84	120
L.S.D. at 5% level:									
Chemicals		2,796		2,760*		528		696*	
Rates		2,364		—		468		—	

* Comparison between columns

TABLE 2.—YIELDS OF BARLEY AND TARTARY BUCKWHEAT, BUSHEL WEIGHT OF BARLEY AND SURVIVAL OF TARTARY BUCKWHEAT PLANTS FOLLOWING TREATMENT WITH VARIOUS HERBICIDES IN 1957

Treatment		Barley				Tartary buckwheat					
		Yield bu./a.		Bu. wt. lb./bu.		Tot. dry wt. lb/a.		Seed yield lb./a.		% survival	
Chemical	Rate	Stage 1*	Stage 2*	Stage 1*	Stage 2*	Stage 1*	Stage 2*	Stage 1*	Stage 2*	Stage 1*	Stage 2*
Check	—	15.2	12.6	—	—	4453	4957	1168	1536	89	94
2,4-D	4 oz./a	45.9	30.2	46.4	45.4	1320	3013	336	708	53	84
2,4-D	8 oz./a	40.9	35.9	45.9	47.2	632	1940	172	480	38	64
2,4-D	12 oz./a	36.7	39.6	45.3	46.1	716	1324	216	336	46	43
LV 2,4-D	4 oz./a	38.2	36.5	46.4	46.8	1468	2849	392	576	60	88
LV 2,4-D	8 oz./a	34.4	34.2	44.5	46.1	448	2409	108	544	32	72
LV 2,4-D	12 oz./a	36.7	36.7	44.6	46.3	648	1248	224	500	25	65
2,4-DB	8 oz./a	45.7	40.9	45.3	46.2	808	1704	288	396	39	59
2,4-DB	16 oz./a	47.5	38.2	45.4	45.1	456	1368	124	220	38	40
2,4-DB	24 oz./a	49.0	41.5	45.3	46.4	372	640	128	232	31	29
Neburon	2.5 lb./a	52.8	37.3	45.1	45.9	680	3685	276	1064	16	86
Neburon	5 lb./a	50.0	32.6	44.3	46.1	0	3013	0	888	0	58
Neburon	10 lb./a	44.2	36.7	44.6	44.6	0	84	0	16	0	1
L.S.D. at 5% level:											
Treatments**		13.6		no significant difference		2076		539		51	
Treatments x Stages		8.1				672		271		10	

*Growth stages of plants at the time of treatment (*see text*)
**Comparison of treatments within one stage

Effects of Competition with or without 2,4-D Treatments

In a greenhouse experiment different numbers of buckwheat seed per pot were planted in triplicate sets of 7-inch pots filled with a 3:1 mixture of black loam and sand. In a study of competition between Tartary buckwheat plants in the field, seed was broadcast on quadruplicate 5′ x 5′ plots, and then covered with approximately 1 inch of soil. Following emergence of seedlings they were thinned by hand to obtain the desired densities. Material was harvested from one set of plots at each of two stages. At the first stage, 68 days after planting, seeds were just beginning to form; at the second stage, 100 days after planting, the majority of the seeds present were mature.

Where the competitive effects of Tartary buckwheat in conjunction with treatments with LV 2,4-D in Gateway barley were investigated in the field, buckwheat was seeded in rows at right-angles to the barley rows with all rows spaced 9 inches. Sufficient buckwheat seed was planted to result in stands of various desired densities, following thinning by hand. Treatments consisted of 1) removal of the weeds by hand, and 2) application of 4 and 8 oz./a. of LV 2,4-D (butoxyethanol ester). The treatments were applied over a 2-day period, 3 weeks after planting. At the time of treatment the barley was approximately 6 inches tall, and had four to five leaves; the buckwheat had two to three true leaves. At harvest time yields of barley and buckwheat were determined.

RESULTS

Comparison of Herbicides, 1956 and 1957

Table 1 shows the fresh-weight and seed-yield data obtained in the fall of 1956. The yield of barley could not be determined on all plots because of bird damage. The results obtained, however, indicated that the yield of a treated plot was in no case lower than that of a weedy check plot. Yields of treated plots ranged from 4 to 37 per cent higher than those of check plots.

Data obtained at harvest time in 1957 are presented in Table 2. The check plot did not yield sufficient grain for the determination of the bushel weight of the barley.

TABLE 3.—EFFECTS OF COMPETITION BETWEEN TARTARY BUCKWHEAT PLANTS GROWN IN POTS IN THE GREENHOUSE

Plants per pot	23 days after planting			72 days after planting		
	Stem diameter, mm.	Maximum leaf width, cm.	Leaves per plant	Fresh wt., gm. per plant	Total seeds per plant	Mature seeds %
1	4.0	6.3	13.0	42.0	373	40.5
3	3.2	5.3	6.5	10.3	98	44.1
5	2.9	4.4	4.5	6.4	56	43.4
10	2.1	4.2	4.5	3.1	28	39.1
15	2.0	3.7	4.0	2.6	21	40.5

TABLE 4.—FRESH AND DRY WEIGHT, AND SEED YIELD OF TARTARY BUCKWHEAT GROWN AT DIFFERENT POPULATION DENSITIES IN THE FIELD

Stage*	Plants per sq. yd.	Total fresh wt.		Total dry wt.		Yield of mature seeds	
		lb./a.	gm./plant	lb./a.	gm./plant	lb./a.	gm./plant
1	25	30,410	114	3,073	11.5	0	—
	50	29,769	56	2,774	5.2	0	—
	100	39,159	37	3,628	3.4	0	—
	200	40,759	19	3,724	1.7	0	—
2	25	25,715	96	5,484	20.6	1,643	6.1
	50	28,382	53	5,815	10.9	1,793	3.4
	100	27,208	26	6,039	5.7	2,347	2.2
	200	26,568	12	5,772	2.7	2,497	1.2

* Growth stage of plants when harvested (*see text*)

Effects of Competition with or without 2,4-D Treatments

Increasing numbers of Tartary buckwheat plants per pot in the greenhouse resulted in reduced stem diameter and leaf width, a smaller number of leaves per plant, and lowered production of vegetative matter and seed per plant (Table 3).

Table 4 shows the results of the field experiment in which Tartary buckwheat was grown at different population densities. With increasing density the plants grew somewhat taller, but they were more spindly and showed less branching.

In Table 5 are summarized the results of the field experiment in which LV 2,4-D was applied at two rates to barley plots infested with different numbers of buckwheat plants per square yard.

Visual observations during the growing season indicated a slight delay in heading, and the failure of a number of barley heads to emerge properly, on plots sprayed with LV 2,4-D at both rates.

DISCUSSION AND CONCLUSIONS

From the experimental results obtained previously by a number of weed workers (4, 5), it is evident that control of Tartary buckwheat by chemical methods is a goal difficult to achieve, and that eradication requires dosages of herbicide which may cause serious injury to the grain.

Examination of the buckwheat data obtained at Edmonton revealed, first of all, a very consistent relationship between fresh weight and seed yield (Table 1) or between total dry weight and seed yield (Table 2). In both cases, therefore, these data will be discussed together. Considering all comparsions in Table 1, it appeared that for control of Tartary buckwheat LV 2,4-D was more effective than 2,4-D, and that both were much superior to MCPA. The difference was most pronounced at the 8 oz./a. rate of first treatment, and where the second treatment was applied on previously untreated plots.

In the 1957 experiment LV 2,4-D and 2,4-D were equally effective in reducing buckwheat growth; LV 2,4-D applied at stage 1 caused more injury to the grain than 2,4-D. The chemical 2,4-DB appeared much less

TABLE 5.—YIELDS OF BARLEY AND TARTARY BUCKWHEAT FROM PLOTS CONTAINING DIFFERENT NUMBERS OF BUCKWHEAT PLANTS PER SQUARE YARD

Treatment	Buckwheat plants per square yard	Barley		Tartary buckwheat		
		Yield, bu./a.	Bushel weight, lb./bu.	Total dry wt., lb./a.	Seed yield, lb./a.	Survival %
Buckwheat plants removed by hand at spraying time	0	69.9	46.2	—	—	—
	25	71.2	44.3	0	0	0
	50	69.7	44.6	0	0	0
	100	57.6	44.5	0	0	0
Check (no treatment)	0	68.6	46.2	—	—	—
	25	64.0	45.0	1387	179	89
	50	49.1	45.1	2185	393	88
	100	27.9	43.6	3271	751	82
LV 2, 4-D at 8 oz./a.	0	62.3	45.0	—	—	—
	25	59.6	45.8	18	2	9
	50	51.9	44.3	75	17	21
	100	43.8	44.4	117	31	16
LV 2,4-D at 16 oz./a.	0	60.1	44.1	—	—	—
	25	51.3	43.6	8	1	3
	50	48.6	43.7	18	5	8
	100	41.3	43.3	55	12	7
L.S.D. at 5% level:						
Treatments*		19.6	1.7	1596	488	9
Densities**		20.8	—	—	492	—
Treatments x Densities		8.3	—	174	75	—

* Comparison of treatments on the same density
** Comparison of densities given the same treatment

injurious to the barley than did 2,4-D, and at 8 oz./a. was as effective as the latter in reducing the buckwheat stand. Highest barley yields and maximum control of buckwheat were obtained following foliage application of neburon at stage 1. Though the results were promising, the cost of this chemical is still prohibitive for practical use. It is of interest that, in a preliminary greenhouse experiment, neburon at rates up to 10 lb./a. was ineffective as a pre-emergence herbicide for the control of Tartary buckwheat.

Repeated applications of herbicide have been reported to be effective in the control of wild buckwheat (*Polygonum convolvulus*) (4). In the case of Tartary buckwheat two separate doses of 4 oz./a. (12 days apart) were less effective than a single application at 8 oz./a. made at the earlier growth stage (Table 1). The importance of spraying the weeds at an early stage is indicated by data in Table 2. Fewer plants survived treatment at the earlier stage, and those that did were stunted much more severely than were survivors of treatment at the second stage.

Competition between Tartary buckwheat plants followed the same trend as that found for other species (7). With increasing number of plants per unit area the total amount of vegetative matter and seed produced increased somewhat, but per plant these values decreased sharply.

In discussing the competition between Tartary buckwheat and barley it must be recognized that the quantitative conclusions drawn should not be construed too generally. It is nevertheless of interest to make some comparisons with findings of other workers. Burrows and Olson (1) found that wild mustard affected the growth of wheat before the latter had reached the five-leaf stage of growth. In the present work the barley had four to five leaves at spraying time; at 100 plants per square yard competition by the buckwheat population during the time before spraying did not cause a statistically significant decrease in the ultimate yield of barley. If the buckwheat plants were present throughout the growing season, grain yield decreased with increasing buckwheat density.

Chemical treatment appeared to decrease, though not to the point of statistical significance, the yield of barley on weed-free plots and on plots with 25 buckwheat plants per square yard, but not on plots more heavily infested with buckwheat. The yield of barley, with increasing buckwheat density, decreased much more rapidly on untreated plots than on plots treated with LV 2,4-D at 8 oz./a. Burrows and Olson (1) concluded that the critical density of wild mustard, at which spraying with 2,4-D could be considered justifiable, depended on the rate of seeding of grain. For buckwheat-infested barley plots sprayed with LV 2,4-D at 8 oz./a. in the present writers' experiment, the critical density appeared to lie between 25 and 50 buckwheat plants per square yard where the barley had been seeded at 1 bushel per acre.

Eradication of Tartary buckwheat by chemical means appears to be impractical at the present time, but with the methods and treatments available it is certainly possible for the farmer to keep this weedy species under control, and to minimize the amount of grain polluted by Tartary buckwheat during harvest operations.

ACKNOWLEDGEMENTS

The financial assistance of the National Research Council is gratefully acknowledged.

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A METHOD FOR ESTIMATING THE PERCENTAGE OF HYBRIDS IN SEEDLOTS OF FIRST GENERATION ADVANCE SUNFLOWERS BY MEANS OF A SEEDLING CHARACTERISTIC[1]

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[Received for publication July 4, 1958]

ABSTRACT

Estimates of the percentage of hybrids and inbreds in seedlots of the sunflower hybrid variety Advance based on the leaf venation of the seedlings were compared with estimates based on mature plant determinations. Agreement between the two estimates was high though not complete. Since the variation in percentage of hybrids which occurs in seedlots of Advance is large, estimates based on seedling characteristics are suggested as a means for evaluating the seed.

INTRODUCTION

The sunflower variety Advance is considered to be a top cross hybrid. The hybrid seed is produced by planting an inbred line (S-37-388) and an open pollinated variety (Sunrise) in alternate groups of two rows each in fields isolated from other sunflowers. Natural crossing occurs in these fields and the percentage of crossing is greater in the inbred (S-37-388) than in the open pollinated variety (Sunrise). The seed from the inbred is harvested and is used as hybrid seed (Advance). Wide variation in the percentage of hybrids is known to occur in commercial seedlots of first generation Advance (2). A difference in the time of flowering of the two parents and the relative abundance of insect pollinators (1) may influence the degree to which cross pollination takes place. The seed yields from seedlots containing low percentages of hybrids cannot be expected to be as high as those from seedlots containing a high percentage of hybrids (3).

The percentage of hybrids in a seedlot can be estimated by growing a sample of plants to maturity and distinguishing the hybrids from the inbreds by their hybrid vigour and other mature plant characteristics. The time and the labour involved almost prohibit the use of this procedure. If seedling characteristics could be used to determine the percentage of hybrids in seedlots, one year's seed could be evaluated before it is used in the next season.

During a search for seedling characteristics which might be added to one or both of the parents of the hybrid, certain differences in the venation of the leaves of the S-37-388 parent and the hybrid were noted. As shown in Figure 1 the venation of S-37-388 is much more reticulate than that of the same leaf of Sunrise or of the cross of the two which is known as Advance. The following tests deal with the possibility of using this characteristic to estimate the percentage of hybrids in seedlots of first generation Advance seed.

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[2] Research Associate.

EXPERIMENTAL METHODS AND RESULTS

Two tests were made in 1953 to compare the results of seedling estimates of hybrids and inbreds based on the differences in leaf venation of S-37-388 and Advance shown in Figure 1, and mature plant determinations based on hybrid vigour and other mature plant characteristics (the usual criteria—head type, seed characteristics, etc.). A commercial seedlot of Advance was used in 1953. In 1954 two sources of seed were used. One of the seedlots was produced at the Experimental Farm at Morden and the other by hand pollination at the University of Manitoba. In all tests plants were grown in check rows spaced 3 feet apart in both directions to facilitate the expression of hybrid vigour. Several S-37-388 checks were included in each test.

In the first test, seed of a commercial seedlot of Advance was sown in the field and later thinned to the required spacing of 3 feet apart in each direction. Four hundred and twenty-two sunflower plants were used in this test. The seedlings were classified as hybrid or inbred on the basis of the differences in leaf venation as illustrated in Figure 1. As the plants approached maturity they were reclassified as either hybrid or inbred on the basis of mature plant characteristics. The results of this test are given in Table 1.

As shown in Table 1 agreement between seedling (42.4 per cent) and mature plant (41.0 per cent) estimates of the percentage of hybrids was good and both estimates indicated that the percentage of hybrids in the seedlot was low.

Soil scattered on the leaves by rain interfered with seedling classification in this test. To circumvent this difficulty 407 seedlings from the same seed source were started in the greenhouse, classified as hybrids or inbreds in the seedling stage and transplanted to the field for mature plant classification. The mature plant classification in this test may be somewhat less reliable than that in the first test due to frost damage which occurred before the plants were fully mature. The results are presented in Table 2.

The mature plant estimates (35.1 per cent) of the percentage of hybrids given in Table 2 are lower than those shown in Table 1 (41.0 per cent). The difference may be due to sampling error or a bias introduced by thinning (i.e. removal of more of the less vigorous plants in the first test). Seedling (32.4 per cent) and mature plant estimates (35.5 per cent) were in reasonably close agreement.

TABLE 1.—A COMPARISON OF SEEDLING AND MATURE PLANT ESTIMATES OF HYBRIDS AND INBREDS IN A SEEDLOT OF ADVANCE

Classification	Mature plant			Seedling totals	Percentage
	Hybrid	Inbred	Doubtful*		
Seedling					
Hybrid	156	23	0	179	42.4
Inbred	17	221	5	243	57.6
Mature plant totals	173	244	5	422	
Percentage	41.0	57.8	1.2		

* Types which could not be classed as S-37-388 or Advance

FIGURE 1. The first true leaves of the sunflower varieties; Sunrise (*left*), Advance and S-37-388 (*right*).

TABLE 2.—A COMPARISON OF SEEDLING AND MATURE PLANT ESTIMATES OF HYBRIDS AND INBREDS IN A SEEDLOT OF ADVANCE

Classification	Mature plant			Seedling totals	Percentage
	Hybrid	Inbred	Doubtful		
Seedling					
Hybrid	124	7	1	132	32.4
Inbred	12	233	11	256	62.9
Doubtful	7	5	7	19	4.7
Mature plant totals	143	245	19	407	
Percentage	35.1	60.2	4.7		

The presence of some plants in the first two tests (Tables 1 and 2) which could not be classified as S-37-388 or Advance in the mature plant stage suggested that better seedling estimates might be obtained with purer samples of Advance seed. In 1954 the purest seed available was used in the test. Two hundred and twenty-nine seedlings of Advance from seed produced at the Experimental Farm at Morden and one hundred and ninety-three seedlings of Advance from seed produced by hand pollination at the University of Manitoba were started in the greenhouse, classified in the seedling stage and transplanted to the field for mature plant determinations. The result of the tests for the seed from Morden and Winnipeg are given in Tables 3 and 4 respectively.

As in the two previous tests the agreement between the estimates from the two methods of determining the percentage of hybrid seed was good.

DISCUSSION

Most of the plants classified as S-37-388 or Advance in the seedling stage were placed in the same group by mature plant determinations (Tables 1 to 4). The percentage of plants placed in the same group by the two determinations were 89.3, 89.4, 93.0 and 96.9 for the respective seed lots and the average for all tests was 91.2.

TABLE 3.—A COMPARISON OF SEEDLING AND MATURE PLANT ESTIMATES OF HYBRIDS AND INBREDS IN A SEEDLOT OF ADVANCE (SEED SOURCE—MORDEN)

Classification	Mature plant		Seedling totals	Percentage
	Hybrid	Inbred		
Seedling				
Hybrid	186	3	189	82.5
Inbred	13	27	40	17.5
Mature plant totals	199	30	229	
Percentage	86.9	13.1		

TABLE 4.—A COMPARISON OF SEEDLING AND MATURE PLANT ESTIMATES OF HYBRIDS AND INBREDS IN A SEEDLOT OF ADVANCE (SEED SOURCE—WINNIPEG)

Classification	Mature plant		Seedling totals	Percentage
	Hybrid	Inbred		
Seedling				
Hybrid	141	0	141	73.1
Inbred	6	46	52	26.9
Mature plant totals	147	46	193	
Percentage	76.2	23.8		

The absence of complete agreement between the two determinations may be related to the degree of variability usually found in a cross-pollinated crop. A small percentage of the S-37-388 seedlings examined were not as clearly reticulate as shown in Figure 1. Hence some S-37-388 plants might be placed in the Advance group by seedling classification. The mature plant characteristics of a few of the S-37-388 plants examined were not typical and hence they might be placed in the Advance group by mature plant determinations. The occurrence of some plants (Tables 1 and 2) which could not be classified as S-37-388 or Advance by mature plant classification is evidence of heterogeneity in the seed which was used in 1953. The high degree of agreement (96.9 per cent) obtained in the last test (Table 4) supports the contention that better agreement could be obtained with purer seed stocks.

The need for a seedling characteristic to estimate the percentage of hybrids in Advance was illustrated by the range (35.1 to 86.9) in the percentage of hybrids of the seedlots used in these tests. Such large differences also indicate that it would be very desirable to have a clearly distinguishable marker characteristic in one of the parents of new sunflower hybrid varieties. Since the variation in the percentage of hybrids in commercial seedlots has been shown to be large, and since the differences between the seedling and mature plant estimates did not exceed 4.4 per cent in any test, seedling classification is suggested as a practical method for estimating the percentage of hybrids in seedlots of Advance.

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CHEMICAL CONTROL OF THE LESSER PEACH TREE BORER, *SYNANTHEDON PICTIPES* (G. & R.) (LEPIDOPTERA:AEGERIIDAE), IN ESSEX COUNTY, ONTARIO[1]

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[Received for publication August 5, 1958]

ABSTRACT

Applications of endrin at 0.90 to 1.02 lb. per acre, or of parathion at 0.27 to 0.31 lb. plus malathion at 1.20 to 1.32 lb., as totals of toxicant from three applications, gave reasonably consistent reductions of the numbers of borer larvae per tree and of reinfested trees during 1955-57. Similar results were obtained with parathion at 0.55 to 0.60 lb. per acre in 1956. In the only season in which it was used dieldrin at 1.03 lb. per acre was less effective than endrin or parathion plus malathion. In 1957 two applications of endrin gave as good results as three. Reduction in the number of borers per tree in each year was good when the first of a series of three seasonal sprays of endrin or of parathion plus malathion was applied at the time that 12 to 29 per cent of the moths had emerged or within 5 to 12 days of completion of treatments against the plum curculio.

INTRODUCTION

Armstrong (1) discussed the seasonal development and injury caused by the lesser peach tree borer, *Synanthedon pictipes* (G. & R.), in the Niagara Peninsula from 1937 to 1941. In addition, he offered suggestions for decreasing the numbers of the borer by using appropriate cultural methods to control peach canker, and by cleaning out and protecting cankered or injured areas with tree wound dressing. Because of the cost of labour, frequently as great as $25.00 per acre, required to clean and dress cankered and infested areas, such measures have been neglected by most growers. As a result the borer has infested 20 to 100 per cent of the trees in various orchards. In many orchards fruit production has been seriously reduced by the premature death of trees caused by the borer and peach canker.

Smith (2) showed that DDT (2,2-Bis(*p*-chlorophenyl)-1,1,1-trichloroethane) was rather ineffective for the control of the lesser peach tree borer, but that three sprays of parathion (*O,O*-Diethyl O-*p*-nitrophenyl phosphorothioate) or of EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate) gave good control.

This is a report on experimental work undertaken in Essex County, Ontario, from 1955 to 1957, to compare the effectiveness of some organic phosphate and chlorinated hydrocarbon insecticides against the borer. The main objective was to develop an effective chemical control, preferably of lower toxic hazard than that associated with the use of parathion.

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MATERIALS AND METHODS

The insecticides used were:

1. *Dieldrin* (1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*exo*-4,8 dimethanonaphthalene).

2. *Endrin* (1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-*endo*-*endo*-dimethanonaphthalene).

3. *Parathion* (*O,O*-Diethyl O-*p*-nitrophenyl phosphorothioate).

4. *Malathion* (*S*-(1,2-Dicarbethoxyethyl)-*O,O*-dimethyl phosphorodithioate).

The formulations used are listed in Table I.

All sprays were applied with a John Bean Spraymaster gun* fitted with a No. 8 disk. The gun was connected with high pressure hose to a Turbo-Mist concentrate sprayer**, the pump being operated at 400 p.s.i.

The insecticides were applied as dilute sprays of wettable powders or emulsions so as to deliver approximately 0.5 gallons of spray mixture per tree per application. The dilution rates per 100 gallons of spray were: emulsifiable endrin, 1 quart; emulsifiable dieldrin, 1 quart; parathion, 15 per cent, 2 pounds.; parathion, 15 per cent, 1 pound, plus malathion, 50 per cent emulsifiable liquid, 1 pint.

The rates of actual toxicant varied as the area to be treated varied from tree to tree and season to season. Accordingly, the gallonage of spray applied per acre varied, but the rates per 100 gallons of the insecticidal formulations did not. The pounds of toxicant applied per acre were calculated from the total gallonage of dilute spray applied per treatment. The sprays were applied to the bases of main scaffold limbs, crotches, and trunks to the ground level to give thorough coverage of those areas that were most severely injured and most critical to the survival of the tree. In all of the experimental areas the tree planting distance was 20 feet each way.

Each insecticide was applied three times in each season except in 1957, when only the first two sprays of endrin were applied to one series of trees. The first applications were made on June 9 in 1955, June 21 in 1956, and June 14 in 1957, when 12, 29, and 16 per cent respectively of the adults had emerged. The second and third applications were made after 3-week intervals to provide coverage for as much of the period of major activity of the insect as was practicable.

The insecticides were applied in randomized blocks, each plot consisting of a single tree. The numbers of replicates were: 25 (in 1955) in a severely infested orchard; 10 (in 1956) in a severely infested, and 12 (in 1956) in a moderately infested orchard; 15 (in 1957) in a moderately infested orchard. Only trees infested with living borers, as determined by examination for fresh frass during May, were treated. Such trees were used to ensure that areas suitable for reinfestation were present because the effect of the treatments was to be evaluated against borers of the current season rather than overwintering borers.

* John Bean Division, Food Chemistry and Chemical Corp., Lansing, Mich.
** Okanagan Turbo Sprayers Ltd., Penticton, B.C.

TABLE 1.—NUMBERS OF THE LESSER PEACH TREE BORER PER TREE IN THE FALL, AND PERCENTAGE OF TREES REINFESTED AFTER THREE APPLICATIONS OF VARIOUS INSECTICIDES AT 3-WEEK INTERVALS, ESSEX COUNTY, 1955-57

Material and total pounds toxicant per acre	Mean number larvae per tree		Trees reinfested, %	Reduction, %	
	Actual	Transformed[1]		Larvae per tree	Reinfested trees
1955, 25 trees per treatment, 12 per cent of moths emerged by first application					
Endrin[2] 0.90	1	1.2831	64	93	36
Parathion[3] 0.31 + malathion[4] 1.32	2	1.5162	72	88	28
Dieldrin[5] 1.03	6	2.4371	96	71	4
Check	21	4.2592	100		
Difference necessary for significance at 1% level		0.8285			
1956, 10 trees per treatment, 29 per cent of moths emerged by first application					
Endrin 1.02	1	1.0500	40	96	55
Parathion 0.30 + malathion 1.20	1	1.1307	50	96	44
Parathion 0.60	1	1.1914	50	95	44
Check	26	4.7633	90		
Difference necessary for significance at 1% level		1.3722			
1956, 12 trees per treatment, 29 per cent of moths emerged by first application					
Endrin 0.93	0.33	0.8472	17	96	82
Parathion 0.27 + malathion 1.17	0.08	0.7502	8	99	91
Parathion 0.55	0.33	0.8662	25	96	73
Check	10.00	2.8194	83		
Difference necessary for significance at 1% level		0.7996			
1957, 15 trees per treatment, 16 per cent of moths emerged by first application					
Parathion 0.27 + malathion 1.17	0.33	0.8800	33	95	61
Endrin 0.93	1.00	1.1333	47	84	46
Endrin 0.64[6]	1.00	1.2460	53	80	38
Check	7.00	2.5533	86		
Difference necessary for significance at 1% level		0.6198			

[1] By the formula $\sqrt{x + 0.5}$.

[2] Endrin-20, an emulsifiable liquid containing 2 lb. technical endrin per gal. Shell Oil Co. of Canada Ltd., Toronto, Ont.

[3] Parathion 15% wettable. Canadian Industries Ltd., and Chipman Chemicals Ltd., Chatham, Ont.

[4] Malathion, 50% emulsifiable liquid containing 5 lb. malathion per gal. Cyanamid of Canada Ltd., Toronto, Ont.

[5] Dieldrin-20, an emulsifiable liquid containing 2 lb. technical dieldrin per gal. Shell Oil Co. of Canada Ltd., Toronto, Ont.

[6] Only first 2 sprays applied.

The materials were evaluated in the fall of each year by examining treated and untreated trees for infestation and by cutting out borers from 10 inches above the crotch to within 2 inches of the ground level. Because of the heterogeneity of the data and the small numbers the data were transformed by the formula $\sqrt{x+0.5}$. The transformed data were submitted to the standard analysis of variance.

The seasonal emergence of adults of the borer from untreated trees adjoining the experimental areas was determined by weekly inspection of infested areas for empty pupal skins, which were removed as recorded.

RESULTS AND DISCUSSION

Effect of Treatments on Borer Larvae

Table 1 shows that each of the treatments significantly reduced the number of borer larvae per tree in heavily or moderately infested orchards. In 1955 dieldrin was significantly less effective than the other materials and it was excluded from the tests in the two following years. In 1956 and 1957 none of the treatments was significantly more effective than the others, even when endrin was used only in the first two applications.

Table 2 shows the weekly emergence of borer adults from untreated trees as indicated by the numbers of empty pupal skins. It is evident from Table 2 that a larger proportion of the seasonal emergence of moths in 1956 had occurred when the first spray was applied than in either 1955 or 1957. The data in Table 1 give no indication that variations of 12 to 29 per cent in moth emergence by the time the first spray was applied influenced the effectiveness of endrin or of parathion plus malathion.

TABLE 2.—WEEKLY AVERAGE NUMBERS OF PUPAL SKINS OF THE LESSER PEACH TREE BORER PER 100 UNTREATED TREES, 1955-57

1955		1956		1957	
Date	No.	Date	No.	Date	No.
May 26	45	May 25	0	May 28	5
June 2	38	June 2	11	June 4	39
9	68	8	80	11	10
16	91	15	147	18	34
23	194	22	89	25	29
30	78	29	110	July 2	61
July 7	154	July 6	92	10	42
14	58	13	101	16	34
21	85	20	49	23	5
28	34	27	61	30	45
Aug. 5	93	Aug. 3	47	Aug. 6	55
11	28	10	67	13	32
18	103	17	81	20	34
25	98	24	36	27	0
Sept. 1	63	31	37	Sept. 3	13
8	49	Sept. 7	42	11	3
15	12	14	7		
22	9	19	9		

In addition to the reduction of the numbers of lesser peach tree borer larvae in infested areas, the treatments reduced the percentage of trees that became reinfested and promoted rapid healing of badly injured areas.

From the aspects of probable hazards of residues on peach fruits at harvest and hazards to spray operators it appears that the combination of wettable parathion at a low rate with malathion emulsifiable liquid may be the most satisfactory to recommend for use by peach growers.

Timing of Applications

According to Smith (2) there is considerable latitude for the timing of sprays for control of the borer in New York State, and application of the first spray when 15 per cent of the adults had emerged gave satisfactory results. The results obtained from the treatments indicate that a similar latitude occurs in Essex County.

From records of the dates of application of sprays applied for the control of the plum curculio and of other sprays applied against the borer it was determined that the first borer spray was applied 12 days after the last curculio spray in 1955, and 5 days after the last curculio spray in 1956 and 1957. According to the tests conducted, satisfactory reduction of populations of the borer may be expected when the first of a series of applications is made during a period of 5 to 12 days following application of the last spray against the plum curculio, and when two more applications against the borer are made at intervals of 3 weeks.

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AN INEXPENSIVE PLANT GROWTH CHAMBER

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[Received for publication August 5, 1958]

The growing of plants for experimental purposes always poses problems for the botanist. Wide variations in day length, light intensity and temperature in a greenhouse precludes the growing of experimental plants under reproducible conditions. Prefabricated equipment for controlling temperature, light and humidity is available only to the more financially fortunate institutions. However, the equipment described in this article has been designed so that the various parts can be added as finances become available, and growth chambers can be started with a moderate outlay for equipment.

During the last 7 years the Department of Botany at the Ontario Agricultural College has been using nine growth chambers in its research program. Five of these chambers have controlled intensity and duration of light, and the temperature is kept within the bounds of normal growth by ventilating with outdoor air when the temperature within the growth chamber reaches a pre-set maximum. The lighting fixture and ventilating fan can be constructed and installed by a local electrical contracting firm for $750.00. Complete control of temperature within the normal growing range can be installed at a later date by means of air-conditioning equipment. Three of the Department of Botany's growth chambers have been equipped in this manner at a further cost of $850.00 each.

With the air-conditioning equipment installed in the chamber, the range of temperature under the lighting fixture can be controlled within plus or minus 2 Fahrenheit degrees over the entire growing area at any required level, and lighting conditions are such that plants can be grown for several generations without showing any ill effects. Tobacco, tomatoes, petunias, common mustard, strawberries, globe amaranth, alsike clover, red clover, peas and beans have all grown well throughout their complete life cycle.

A number of botanists have shown interest in these chambers, and it is believed that others may be looking for such a relatively inexpensive but efficient method of growing experimental materials.

Figure 1 is a photographic view into a chamber and shows the basic features. Each chamber is 11 feet long, 9 feet wide and 8 feet high. The room was available in the building and only the studding and plywood for walls and ceilings were required. The plywood was backed with aluminium foil for insulation, and painted white to increase reflection.

The lighting unit is 8 feet long and 5 feet wide and consists of 28 daylight slimline fluorescent tubes No. 96T8 (F), supplemented by 10 incandescent 60-watt reflector bulbs (B). This combination of fluorescent tubes

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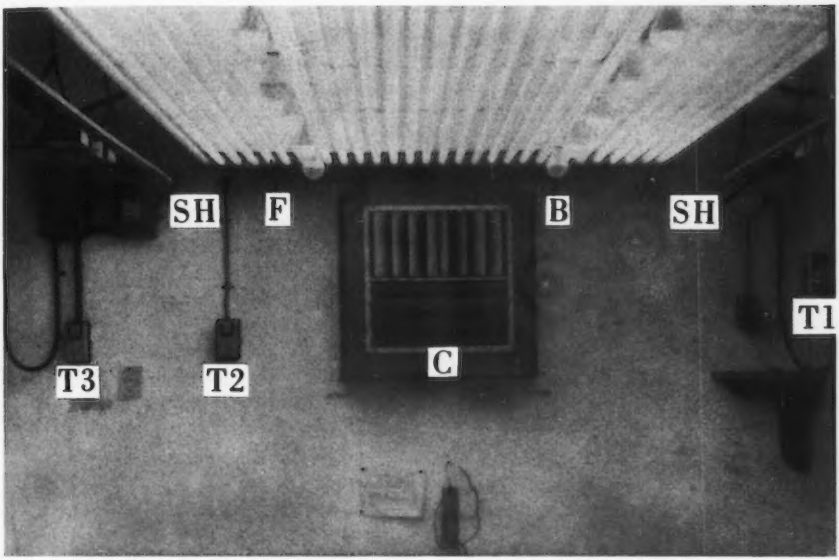

FIGURE 1.—Arrangement of equipment in a growth chamber.

F — Fluorescent Tubes
B — 60 Watt Reflector Bulb
SH — Strip Heater
C — Air Circulation Unit
T1 — Temperature Control
T2 — Light Panel Control
T3 — Strip Heater Control

and bulbs gives a well-balanced light. Using aluminium foil side drapes, the illumination averages 1800 foot candles over the bench at a level of 2 feet below the lights.

In the first three panels constructed, General Electric 59G464 parallel-wound ballasts were used. General Electric discontinued this type, and Sylvania-Sola 608-407 series-wound ballasts were used in later chambers. Both makes of ballasts are designed for the 96T12 fluorescent tubes, but when used in conjunction with the T8 tubes these ballasts give a 50 per cent increase in intensity with only a 70 per cent increase in power used. The only possible disadvantage that we observed in using T8 tubes under these operating conditions instead of the T12 is in their shorter life.

Refrigeration for temperature control in each chamber is supplied by a 2 h.p. sealed, 230 V. Freon 12 water-cooled unit which has more than enough cooling capacity to compensate for the heat from the lighting unit and from the outside, even during hot summer days.

The air circulation unit (C)* consists of a 1/12 h.p. motor geared to a high capacity low speed fan. It is a low differential type with the coil temperature never going more than 10 Centigrade degrees below the temperature set on the thermostat. Air is recirculated approximately three times per minute. Cool air is released so that a portion of it is discharged along the ceiling over the light panels, and the remainder is directed toward the sides of the chamber. The cross-sectional area of the return vent, near the floor under the inlet, should be such that it will carry the stream of air without resistance. No provision for the introduction of fresh air into the chambers was found necessary because the leaks around the door and heat exchanger provided an adequate supply of fresh air.

The time of illumination is automatically controlled by time-clocks and when the lights are cut off, the heat load against the refrigeration unit is maintained by the automatic cutting in of four 750-watt strip heaters (SH).

The thermostats used in the circuits are the Minneapolis Honeywell type T42A1X3 and give plus or minus 2 Fahrenheit degrees control at bench level; usually they are better than this average. Three thermostatic controls are used; T_1 regulates the refrigeration cycle; T_2 is a safety regulator which trips off the lights should the refrigeration unit fail to function; T_3 cuts off the strip heaters in the event of refrigeration failure.

There is a line of three complete chambers, each of which is kept at a different temperature. The plants are on movable carts and may be wheeled from one chamber to another.

The mechanical and electrical components used in these growth chambers are of standard make and their operation has been thoroughly tested in commerce. The operation of the chambers has been found to be very reliable and no major breakdown has been experienced in 7 years. The operating characteristics will be maintained for months with no attention except for lubrication and the replacement of an occasional incandescent lamp or fluorescent tube.

* Air Coils Mfg. Co., Oakville, Ont.

RHIZOCTONIA RUBI SP. NOV. ASSOCIATED WITH THE DRY-BERRY DISEASE OF THE LOGANBERRY

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[Received for publication November 17, 1958]

ABSTRACT

Rhizoctonia rubi sp. nov., which was isolated from incipient lesions on loganberry and Boysenberry dry-berry fruit, is described. This fungus was obtained, without exception, from diseased fruit of both hosts and for several reasons is believed to be the primary cause of the destructive dry-berry disease on the Pacific coast of North America. The history, importance and symptoms of the dry-berry disease are given. This disease is clearly distinguished from anther and stigma blight, caused by *Haplosphaeria deformans*, with which it has been confused.

INTRODUCTION

Fruit blight disease of loganberry, which is commonly known as dry-berry in the Pacific North West is an extremely destructive disease on Vancouver Island, British Columbia. In 1928, a 50 per cent crop loss was reported by Foster (13), and in 1940 Jones (6) estimated that 30 per cent of the crop was destroyed. Equally severe epidemics have occurred in several other years (1, 7). However, some years trace amounts only of this disease are present, or only a few fields are severely infected (4).

Because of the seriousness of dry-berry in the absence of any control measures, and since doubt existed as to the cause of this disease, an investigation was undertaken.

REVIEW OF LITERATURE

In 1931, Foster (11) described a bacterial fruit blight of loganberry and claimed that *Bacillus desiccans* was the primary causal organism. A little later, Newton (3) indicated that thrips, not *B. desiccans*, were the primary cause but that thrips alone were unable to produce any serious damage. Other bacterial isolates were more pathogenic than *B. desiccans*. In 1933, Dearness and Foster described anther and stigma blight of loganberry and showed that it was caused by *Haplosphaeria deformans* (10). Since 1935, Foster (4, 5) and others (6) have assumed that dry-berry and anther and stigma blight were synonymous and that *H. deformans* was the causal organism.

In 1945, Breakey (1) stated that the cause of the dry-berry disease had not been established but he thought that the mite *Phyllocapta gracilis* was associated with dry-berry. He stated: "Perhaps the mere presence of the mite is not enough to prove that it is the cause of the dry-berry disease. It can be stated, however, that all known facts support this assumption."

In 1953, the author (7) stated that anther and stigma blight which is caused by *Haplosphaeria deformans* is distinct from the dry-berry disease.

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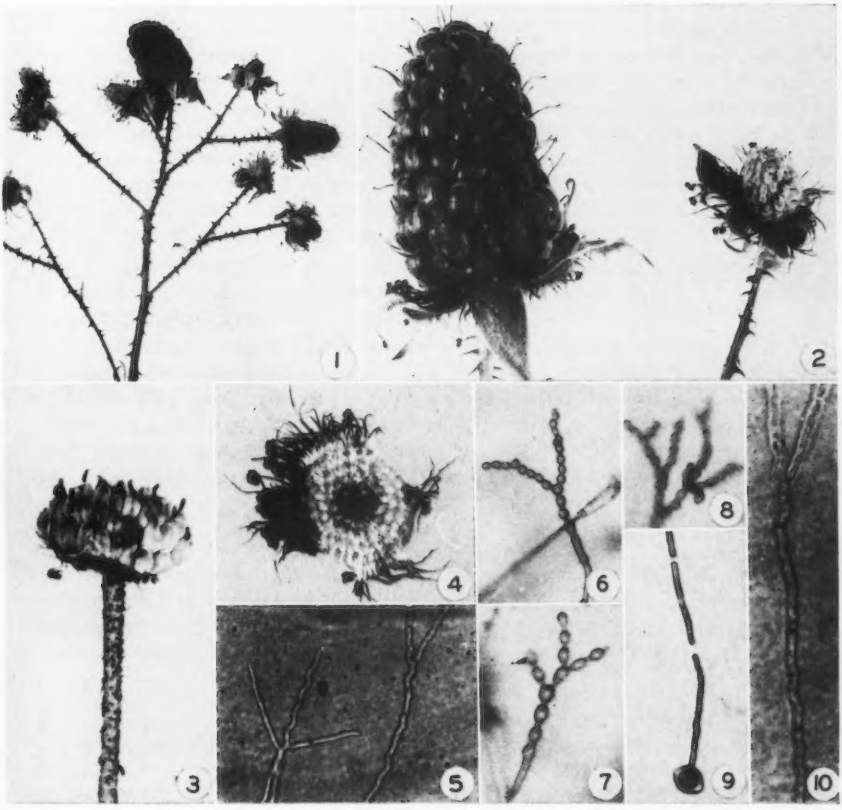

FIGURES 1–10.

FIGURE 1. A Loganberry fruit spur with diseased and healthy berries. Observe that only the upper portion of the pedicels become diseased.

FIGURE 2. A healthy and diseased loganberry; note the bleached and dry nature of the diseased fruit.

FIGURE 3. A thimbleberry fruit soon after infection. Only one druplet is blackened.

FIGURE 4. A thimbleberry receptacle which is necrotic in the central region.

FIGURE 5. Typical branching mycelium of *Rhizoctonia*.

FIGURES 6, 7, 8. Branched chains of pseudo-spores.

FIGURE 9. A pseudo-spore and germ tube.

FIGURE 10. Septate mycelium.

SYMPTOMS

Berries affected by physiological drought are quite distinct from those suffering from the disease known as dry-berry. Berries do not show disease symptoms of dry-berry until they are half their mature size. In the green berries a brown discoloration of a druplet begins at the base of the style and very rapidly spreads through the druplet into the receptacle and base of the adjoining druplets. Within 24 hours after the first symptoms the whole fruit aggregate is necrotic, dry and crumbly. The druplets become dry, shrunken and separated from each other and cling to the receptacle for a long time. Necrosis usually spreads down the pedicel from one-quarter to one inch. Progress of disease in red unripe berries is similar to that in green berries and the fruit becomes faded, dry and flaccid (Figures 1, 2). Similar symptoms also occur on the wild thimbleberry (Figures 3, 4).

Usually the disease becomes apparent in one or two peak periods of 1- and 2-day duration each season during June or early July. It does not appear to spread from fruit to fruit.

Anther and stigma blight occurs earlier in the season before and immediately after the flowers open. The white mycelium and black pycnidia of *Haplosphaeria deformans* are readily observed on the anther and stigma. Usually, a few druplets are attacked and do not develop, and a malformed fruit results. Sometimes the whole fruit is diseased. The cascadeberry and boysenberry are more susceptible than the loganberry.

ISOLATION

Numerous isolations were made from incipiently-infected druplets, receptacles and pedicels of both loganberry and thimbleberry during the seasons 1953-55 inclusively. Without exception the same slow-growing fungus emerged from the infected tissue.

DESCRIPTION

Mycelium in cultura Rhizoctonia simile (Figures 5, 10). Ramusculi laterales ad iuncturas more solito constricti: septo primo 10 μ-55 μ a iunctura distante. Hyphae aeriae albo primo colore, deinde fusco vel luteo. Hyphae vegetatae in mediis primo sine colore, deinde fuscae, nonnunquam flexuosae, 4.5-7 μ, nonnunquam 13 μ. A latere aliae hyphae augescunt angulo saepe 90°, saepius tamen 45°; primo curvantes, deinde linea parallela cum hyphis parentibus crescentes. Augescit tardius mycelium (Figure 11), optimo temperaturae inferiore quam R. Solani (14). Sunt pseudosporae (Figures 6, 7, 8, 9) 8.6-4.3 μ × 11.8-7.5 μ in catenis ramosis longisque, quae monilibus similes esse videntur, in sporodochiis. Triginta vel triginta quinque diebus sclerotia ad ½ cm. longitudine, nonnihil depressa, nigro colore, subglobosa ad elongata, ab hyphis robustis curtisque formantur, quorum cellulae curtae cadulisque prope sunt similes.

Mycelium in culture *Rhizoctonia*-like (Figures 5, 10). Lateral branching characteristically constricted at the point of union, with the first septum 10 μ to 55 μ from the point of junction. Aerial hyphae white at first, later becoming brown or yellow. Vegetative hyphae in the media colourless at first, later becoming brown, sometimes flexuous, 4.5 to 7 μ occasionally 13 μ. Lateral growth often at right-angles but more commonly making a 45-degree angle with, then curving and growing parallel to the parent hyphae. Mycelium grows at a slow rate and has a lower temperature optimum (Figure 11)

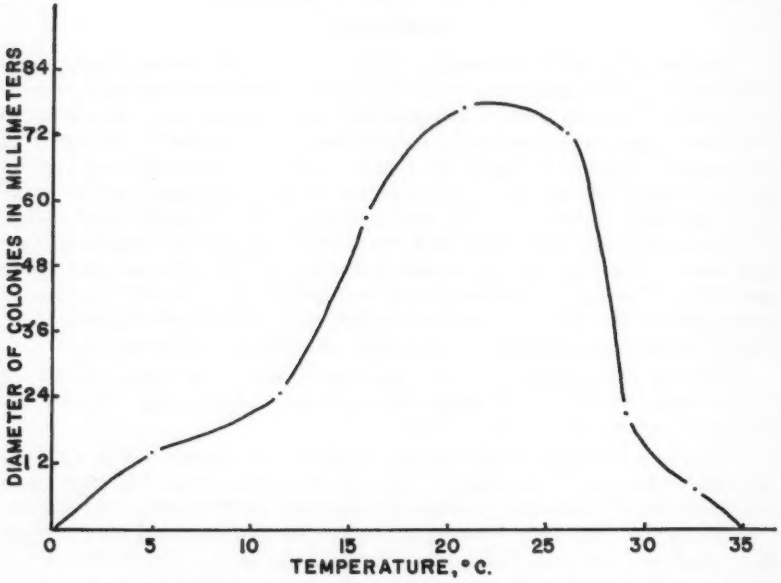

FIGURE 11. Influence of temperature on the rate of growth of *Rhizoctonia rubi*.

than *R solani* (14). Pseudo-spores (Figures 6, 7, 8, 9) 8.6–4.3 x 11.8-7.5 μ occur in long, branched monilia-like chains in sporodochia. After 4 or 5 weeks, slightly embedded black subglobose to elongate sclerotia up to $\frac{1}{2}$ cm. in length are formed from short stout hyphae, the cells of which are short and nearly barrel shaped.

On fruits of loganberry [*Rubus Loganobaccus* (Bailey)] and wild thimbleberry [*Rubus parviflorus* (Nutt)].

Culture is deposited in the American Type culture collection, Washington, D.C., United States, and the Central-Bureau voor Schmmelcultures, Baarn, Holland.

R. rubi is readily distinguished from *R. solani* by its compactness, presence of pseudo-spores, absence of fern-like growth, slower rate of growth and lower temperature optimum.

It is distinguished from the orchid species of *Rhizoctonia* described by Curtis (9) by the presence of sclerotia and usually smaller size of the pseudospores. It is quite different from the Italian species described by Sappa and Mosca (16, 17) and Castellan (2) which produce chlamydospores or pseudoconidia. It is unlike the *Rhizoctonias* of Puerto Rico (12), or the *Rhizoctonia* on corn (18), *Elaeagnus pungens* (19), carrot (15) or coffee (8) and other described *Rhizoctonias*.

Fruits inoculated with this fungus produced typical symptoms but a few of the uninoculated fruit which had been covered with plastic bags also became infected with this pathogen. *R. rubi* was isolated from these diseased control fruits as well as from those that were artificially infected.

DISCUSSION

The fact that some of the covered control fruits suffered from dry-berry indicates that *R. rubi* must have been present on the control fruit when they were covered but was unable to produce disease until the fruit had reached a certain stage in its maturity. Field observations show that only fruit in later stages of development show disease symptoms.

It is probable that *R. rubi* becomes established in a dead or wounded stigma or style and subsequent development is dependent on favourable conditions. Once this fungus has gained entrance into the druplet it becomes very pathogenic. The dry rot is typical of that which could be produced by a species of *Rhizoctonia*.

The fact that *R. rubi* was the only organism present in incipient lesions of both the loganberry and thimbleberry fruit supports the hypothesis that this fungus is the primary cause of dry-berry.

ACKNOWLEDGEMENT

Grateful acknowledgement is extended to J. D. Ralph, Head of the Department of Classics, University of Western Ontario, London, Ont., for translating the fungus description into Latin.

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SOIL INSECTICIDES FOR CONTROL OF ROOT WEEVILS IN STRAWBERRIES IN BRITISH COLUMBIA[1]

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[Received for publication July 8, 1958]

ABSTRACT

On a light, gravelly, high-mineral soil in southern Vancouver Island, each of the following treatments gave satisfactory protection from the black vine weevil, *Brachyrhinus sulcatus* (F.), and the strawberry root weevil, *B. ovatus* (L.), for the 3½-year life of the strawberry planting: a pre-planting soil application of aldrin at 5 lb., dieldrin at 3 lb., or chlordane at 10 lb. toxicant per acre as a dust, in combination with an application to the transplant roots at 5 lb. of 2½, 1½, or 5 per cent dust respectively per 10,000 plants. Three foliage applications, each made at 30 lb. of the appropriate dust per acre during the first 2 years, were not necessary to give protection against the larvae when the soil and roots were treated. The numbers of *B. sulcatus* larvae per plant and the yields in tons per acre from the treated plots in the third crop year averaged 0 and 2.8, in comparison with 11.6 and 1.1 from the untreated plots. In the latter, many of the plants were killed by *B. sulcatus* larvae. Applications of 2½ per cent aldrin dust to the soil, roots, and foliage, to the soil and roots, and to the soil alone gave an average yield per acre in the third year of 1.9 tons in comparison with 1.1 tons when applied to the roots alone or .02 tons from untreated plots. The treatments were not effective against the obscure strawberry root weevil, *Sciopithes obscurus* Horn, and weevils of the genus *Nemocestes* [mainly *N. incomptus* (Horn)].

INTRODUCTION

The black vine weevil, *Brachyrhinus sulcatus* (F.), although present in strawberry plantings in British Columbia for many years (6), was not considered a serious pest until 1950 and became a major problem by 1952 (3). Before *B. sulcatus* became abundant the strawberry root weevil, *B. ovatus* (L.), was the major pest of strawberries and was controlled by two or more applications of root weevil bait, consisting of dried apple waste and sodium fluosilicate. However, *B. sulcatus* was not readily attracted to this bait and many survived (2). Also, after about 1948, many growers began using overhead irrigation along with a sawdust mulch; both created higher humidity and ideal incubation sites for weevil eggs. At the Victoria laboratory, *B. sulcatus* lived longer and laid more eggs which had a shorter incubation period and a higher percentage hatch at 75 to 85 per cent than at 50 to 60 per cent relative humidity.

A 2-year-old strawberry planting at the Renny Farm, Saanich, British Columbia, was destroyed in 1952 by the larvae of *B. sulcatus*. This outbreak, the first of several in this area, occurred on light, gravelly, high-mineral soil (pH 5.8) treated before planting with BHC at 1 lb. of the gamma isomer per acre for control of the June beetle *Polyphylla perversa* Csy. (1). The planting was mulched with 3 to 4 inches of sawdust, received overhead irrigation, and was properly baited for control of *B. ovatus*. There was an average of 18 black vine weevil larvae per plant.

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In 1953, Neiswander (5) found aldrin, dieldrin, and heptachlor, each at 10 lb. toxicant per acre, effective against *B. sulcatus* in plantings of various species of *Taxus*, but chlordane at the same rate was ineffective. In 1955, Eide (4) also found aldrin, dieldrin, and heptachlor effective and chlordane ineffective against both *B. sulcatus* and *B. ovatus* when used as pre-planting soil applications in strawberry fields.

This is a report on an experiment begun in 1953 on Vancouver Island, British Columbia, where insecticides were applied as dusts to the soil before planting, to the roots of strawberry transplants, and to the foliage, in an attempt to obtain protection from weevil attack for three cropping seasons. The effectiveness of treatments with aldrin dust applied to the soil and roots, soil alone, and roots alone is also reported.

MATERIALS AND METHODS

In April, 1953, in a half-acre field near the infested planting on the Renny Farm, plots were arranged in five randomized blocks, each containing three plots, 4 yards (four rows) wide, for treatments and one plot, 2 yards (two rows) wide, for an untreated check. All plots were 32 yards long. An 18-inch fertilizer spreader was used to broadcast the dusts which were immediately mixed to a depth of 6 inches in the soil with a 4-foot rotovator. The roots of transplants, of the variety British Sovereign, were treated with appropriate dusts by means of a salt-shaker applicator (2) and planted in the designated plots. This method of root treatment gave immediate mortality of *P. perversa* larvae in earlier tests (2) and was not phytotoxic. Foliage applications of dusts were made just before oviposition by *B. sulcatus* began in 1953 and 1954 but these were discontinued in 1954 to allow adults to enter the field and oviposit freely and thereby test the effectiveness of the soil and root treatments alone against the larvae as they entered the root area. Table 1 shows the insecticides used and the rates and dates of application. Four inches of sawdust was spread over the planting in the fall of 1953 to conserve moisture and suppress weeds. Overhead irrigation was applied as required.

Serious weevil damage was not apparent in the untreated plots until the spring of 1956. At this time, three plants were preselected at random from the two rows of each untreated plot and from the centre two rows of the treated plots in three of the five blocks; the soil in a circle 12 inches in diameter around each plant, by 6 inches deep, was excavated and sifted for larvae. Small larvae were not separated to species but adults were observed when they emerged. Yields were recorded for each plot in 1956, the third and last cropping season, by taking the total weight of fruit picked from one side of the centre two rows of each plot and expressing this as the weight of fruit per row of 65 plants.

Alongside the randomized blocks a 5–x–5 latin square, consisting of single-row plots of 12 plants each, was set up in 1953. In this test, 2½ per cent aldrin dust was applied in four ways, namely: to the soil only; to the transplant roots only; to the soil and roots; and to the soil and roots plus foliage applications. The soil was treated in a 15-inch band and the dust worked in to a depth of 6 inches with a 15-inch Howard rotovator. Table

TABLE 1.—AVERAGE NUMBERS OF ROOT WEEVIL LARVAE PER PLANT AND AVERAGE STRAWBERRY YIELDS IN 1956 AFTER APPLICATION OF DUSTS TO THE SOIL AND ROOTS[1] BEFORE PLANTING IN 1953[2]

Insecticide dusts	Toxicant per acre (lb.)	Larvae			Yield	
		Number of plants examined	*B. sulcatus*	Other species[5]	Pounds per 65 plants	Equivalent tons per acre
Aldrin, 2½[3] per cent	5	9	0	0.3	41.3	3.0
Dieldrin, 1½[3] per cent	3	9	0	1.0	38.3	2.9
Chlordane, 5[4] per cent	10	9	0	10.4	32.8	2.5
Untreated		15	11.6	13.1	14.8	1.1
Difference necessary for significance at 5% level			—	—	12.9	—

[1] Roots treated at 5 lb. of appropriate dust per 10,000 plants
[2] Foliage treated at 30 lb. of appropriate dust per acre on July 10, 1953, and May 2 and July 24, 1954
[3] Julius Hyman & Co., Denver, Colo.
[4] Velsicol Corp., Chicago, Ill.
[5] Larvae not separated, but adults nearly all of *S. obscurus*

2 shows the rates and dates of application. In May, 1956, one plant preselected at random from each plot was dug and soil examined for weevil larvae. Small larvae were not separated to species. The total weight of fruit from each plot was recorded in 1956. One plant per plot was examined for larvae in March, 1957. Small larvae were compared with known species and separated.

RESULTS

Table 1 shows that, in the experiment with the three chemicals, no *B. sulcatus* larvae were taken in 1956, the third crop year, from any of the treated plots, but an average of 11.6 per plant were taken in the untreated ones. Larvae of other species, mainly the obscure strawberry root weevil, *Sciopithes obscurus* Horn, were collected from both treated and untreated plots but chiefly from the chlordane plot in Block 5 and the untreated plot in Block 1. *B. ovatus* larvae were also taken in the untreated plots. Yields were significantly higher for all treatments than for the untreated plots (Table 1). No treatments were phytotoxic.

Table 2 shows that *B. sulcatus* larvae were present in the third crop year in all plots treated by various methods with aldrin dust, and no significant differences occurred. Nearly all plants in the untreated plots were killed by *B. sulcatus* larvae by 1956. Of the other species taken, *S. obscurus* was the most abundant, significantly more being taken from the plots in which only the roots were treated than from the others. Significantly less fruit was picked from the plots in which only the roots were treated than from other treated plots.

Table 3 shows that all the aldrin dust treatments were less effective in 1957 than in 1956 (Table 2) against *B. sulcatus*. Other species present in all treated plots were *B. ovatus*, *S. obscurus* and *Nemocestes* sp., but there were no significant differences between the treatments.

TABLE 2.—AVERAGE NUMBERS OF ROOT WEEVIL LARVAE PER PLANT[1] AND AVERAGE STRAWBERRY YIELDS PER PLOT IN 1956 AFTER APPLICATION OF 2½ PER CENT ALDRIN DUST[2] BY VARIOUS METHODS IN 1953

Application method	Larvae		Yield	
	B. sulcatus	Other species[5]	Ounces per 12 plants	Equivalent tons per acre
Soil + root[3]	0.6	8.0	80	2.0
Soil + root + foliage[4]	2.0	2.0	76	1.9
Soil only	2.8	7.2	71	1.8
Root only	6.6	36.2	42	1.1
Untreated	9.2	2.2	1	0.02
Difference necessary for significance at 5% level	—	17.4	24	—
at 1% level	—	—	33	—

[1] One plant examined in each of 5 replicates
[2] Julius Hyman & Co., Denver, Colo.
[3] Aldrin applied to soil in 15-in. band to rows 3 ft. apart at 2 lb. toxicant per acre and rotovated to a depth of 6 in., and to roots at 5 lb. of 2½% dust per 10,000 plants
[4] Foliage treated at 30 lb. of dust per acre on July 10, 1953, and May 2 and July 24, 1954
[5] Larvae not separated, but adults nearly all of *S. obscurus*.

TABLE 3.—AVERAGE NUMBERS OF ROOT WEEVIL LARVAE PER PLANT IN 1957 AFTER APPLICATION OF 2½ PER CENT ALDRIN DUST BY DIFFERENT METHODS IN 1953

Application method[1]	*B. sulcatus*	*B. ovatus*	*S. obscurus*	*Nemocestes* sp.
Soil + root	2.4	1.0	25.4	2.8
Soil + root + foliage	2.8	1.6	9.0	3.4
Soil only	5.6	1.8	36.6	2.2
Root only	16.2	4.0	9.4	1.0

[1] See Table 2 for methods and rates. No untreated plants were examined as all were killed by *B. sulcatus* larvae.

DISCUSSION

On Vancouver Island, untreated strawberry plants are damaged or killed by root weevils before the second or, with high populations of weevils, before the first cropping season. In these tests, the failure of *B. sulcatus* and *B. ovatus* to increase in the untreated plots until 1956, the third crop year, was undoubtedly a result of high adult mortality from application of dusts to foliage of adjoining plots in 1953 and 1954. These foliage treatments killed adults that normally would have invaded the untreated plots, laid eggs, and caused severe larval damage to the roots in 1954 and 1955. Since larvae of *Brachyrhinus* weevils were not found in 1956 in the treated plots but were found in the untreated plots (Table 1), the previous applications of dusts to the foliage, although very effective against adults feeding on the foliage, were not necessary to give protection against the larvae when the soil and roots were treated.

In the third crop, the untreated plots yielded about a third as much as the aldrin and dieldrin plots, and half as much as the chlordane plots (Table 1). At 1956 prices (24 cents per lb.) the loss in cash receipts from the untreated plots as compared with the aldrin plots was $912.00 per acre. If no foliage treatment had been applied, this loss probably would have occurred in the second crop, and the untreated plants ploughed under, thereby giving one and one-third crops instead of the expected three crops. Based on an average yield of 3 tons per acre at 1956 prices, this loss would have been $2,352.00 per acre.

The concentrated area of small plots was used in the 5-x-5 latin square test to ensure that all plots would be more uniformly subjected to infestation, as these pests do not fly. Yield data proved superior to larval counts from one plant per plot for measuring the effectiveness of the treatments against *Brachyrhinus* weevils (Table 2), but the examination of more plants per plot might have given significant differences in the larval counts. Also, the lack of greater differences between the numbers of *B. sulcatus* larvae taken from untreated plots and those from treated ones was caused by the movement of some of the larvae away from the untreated plots after they had killed the plants. Root and crown damage to the dead plants was typical of that caused by *B. sulcatus* larvae.

Although there was no significant difference between the yields from plots in which only the soil was treated and from those in which both the soil and roots were treated, the latter treatment is recommended for light, stony soil which is usually heavily infested with *P. perversa* larvae in southern Vancouver Island.

The tolerance of *S. obscurus* and *Nemocestes* sp. larvae to the soil insecticides aldrin, dieldrin, and chlordane, indicated in these experiments, has been verified in more recent tests at Victoria. These results are to be published shortly.

ACKNOWLEDGEMENTS

The authors are indebted to J. Raine, Assistant Entomologist, and B. D. Ainscough, Student Assistant at the Victoria laboratory, for their assistance in applying materials and collecting data. The co-operation of Mrs. N. Renny and Miss B. Renny, on whose farm the tests were conducted, is gratefully acknowledged.

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EFFECTS OF VARIOUS LEVELS OF CALCIUM, MAGNESIUM, AND SULPHUR IN NUTRIENT SOLUTION ON FECUNDITY OF THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS TELARIUS* (L.) (ACARINA: TETRANYCHIDAE), REARED ON CUCUMBER[1]

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[Received for publication September 3, 1958]

ABSTRACT

Progeny of the two-spotted spider mite, *Tetranychus telarius* (L.), reared on cucumber plants grown on a vermiculite base at three levels of calcium, magnesium, and sulphur decreased linearly as dosage of calcium was increased. The average number that developed per female in 35 days on plants receiving 320 mg. of calcium per litre of nutrient solution was 39 per cent less than for plants receiving 160 mg. The results suggested a linear increase in the number of progeny when the level of sulphur was increased from 192 mg. per litre to 288 and 384, and that the number of progeny was greater at 42 mg. of magnesium per litre than at 28 or 56. Concentrations of the three levels of calcium, magnesium, and sulphur supplied were calculated and not confirmed by analysis. The minor element concentrations were not varied. Statistically the experiment was set up as a 3-x-3-x-3 factorial confounded in blocks of nine units, the second-order interaction being partially confounded. There were three replications, each having 27 treatment combinations. No foliage analysis was carried out. The results indicate that, if calcium were used at appropriate levels in greenhouse fertilizers, two-spotted spider mite populations on cucumber would be kept at lower densities.

INTRODUCTION

Various levels of calcium, magnesium, and sulphur were fed to cucumber plants at the Harrow laboratory during the 1952 growing season to determine effects on fecundity of the two-spotted spider mite, *Tetranychus telarius* (L.). This was the second part of a study on effects on the numbers of progeny of single female mites when nutritional mineral elements essential to their metabolism are varied through the intermediary of the cucumber host.

The literature on effects of plant sap on feeding and reproduction of the mites was reviewed in a previous paper (4).

MATERIALS AND METHODS

Burpee hybrid cucumber plants were used throughout. Three seeds were planted per pot; and at the cotyledon stage, approximately one week after planting, plants of uniform size were retained, one per pot, and all others were discarded. Clay pots, 6 inches in diameter with half-inch drainage holes, afforded sufficient space for the extensive root system developed by the cucumber plants.

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All pots were filled with vermiculite. A spectrographic analysis of this material (4) revealed the presence of Si, Fe, Ca, Mn, K, and Na. A chemical analysis[1] showed from 40 to 210 p.p.m. of water-soluble Mg as well as 0.4 to 0.5 per cent Mg soluble in 1 per cent HCL, the latter fraction being considered to be slowly soluble in nutrient solution. Cucumber plants grown for 8 weeks on a vermiculite base, with distilled water only added, developed normally on their cotyledon food reserves to a height of 4 inches, reached the first-leaf stage (2 weeks after seeding), but failed to form the second leaf. From the second week onwards the plants became stunted, showed external symptoms of severe nitrogen deficiency (2) and eventually died. Plants grown on vermiculite for a similar period but fed the standard nutrient solution level from the first-leaf stage showed lush leaf growth, developed to a height of 3 feet 6 inches, and reached the 15-leaf stage. It appeared from the tests that elements present in vermiculite were in soluble amounts insufficient to become a factor in an experiment of this nature.

Stock solutions of nutrients were prepared as recommended by Hoagland and Arnon (3). Six of these contained molar concentrations of one of the following salts: Ca $(NO_3)_2$, K_2SO_4, $NH_4H_2PO_4$, KNO_3, and $NaNO_3$. A seventh solution contained minor elements: 0.5 p.p.m. of B and Mn, 0.2 p.p.m. of Cu and 0.01 p.p.m. of Mo. An eighth solution contained 0.5 per cent (w/v) of iron tartrate. From these stock solutions a series of nutrient solutions were prepared on the basis of a 3-x-3-x-3 factorial experiment, i.e., three replications of the three principal elements Ca, Mg, and S, each at three concentrations, were tested in all possible combinations. The concentrations were 160, 240, and 320 mg. of Ca/litre; 28, 42, and 56 mg. of Mg/litre; and 192, 288, and 384 mg. of S/litre. These concentrations were calculated and not confirmed by analysis. All nutrient solutions contained approximately 704 mg. of K and 322 mg. of N.

At the beginning of the experiment, 4 litres of each mixture were prepared and the pH was adjusted to 6 by the method of Spurway (6). These mixtures were stored in darkened cabinets when not in use.

Sufficient and equal amounts of nutrient mixture were added to each pot weekly to maintain growth conditions; i.e. growth of plants receiving nutrient solution was maintained at a rate similar to that of plants grown in greenhouse soils. Plants were watered daily with distilled water. Loss by drainage was avoided at all times.

Specimens of the spider mite used as the test animal were identified[2] as of *Tetranychus telarius* (L.).

One quiescent deutonymph female and three adult males were placed on each plant at the first-leaf stage, that is, approximately 1 week after the cotyledon stage. These were obtained from an inbred stock that had been isolated for 3 months before its use. All females were fertilized, as was indicated by the presence of both sexes in the progeny on each plant. No plant or female was lost. Pertinent to the conclusion reached in paragraph one, "Discussion and Conclusions", it was observed that mites of the inbred stock reared on Burpee hybrid cucumber in greenhouse soils at the

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Harrow laboratory did not differ significantly in their reaction (i.e., population increases and decreases) to the fertilization program from mites developing on the same variety and program in privately-owned greenhouses. This was expected since each greenhouse mite population is, in effect, an inbred stock.

Cellulose adhesive tape, 1 inch wide, was applied around the outside of each pot at the top, and a half-inch barrier of white petroleum jelly was applied to the centre of the tape to prevent foreign mites from gaining access to the host plant, and to prevent the movement of the test animals from plant to plant.

The experiment was set up as a 3-x-3-x-3 factorial in blocks of nine units, the second-order interaction (CaMgS) being partially confounded (1). Each replicate had 27 treatments, comprising all possible combinations of the three levels of calcium, magnesium, and sulphur [levels of each element hereinafter numerically designated as "1" for the first level, "2" for the second, and "3" for the third (Table 1)]. There were three replications. Replicates were started at weekly intervals in June and were concluded at weekly intervals 7 weeks later.

TABLE 1.—TOTAL NUMBERS OF *T. telarius* (EGGS, NYMPHS, ADULTS) THAT DEVELOPED FROM ONE FEMALE DEUTONYMPH IN 35 DAYS ON SINGLE CUCUMBER PLANTS GROWN ON DIFFERENT LEVELS OF CALCIUM (Ca), MAGNESIUM (Mg), AND SULPHUR (S)[1]

$Ca_1Mg_1S_1$[2]	5328[3]	$Ca_2Mg_1S_1$	3459	$Ca_3Mg_1S_1$	3325	(Mg_1S_1)	*4037*
$Ca_1Mg_1S_2$	5723	$Ca_2Mg_1S_2$	2208	$Ca_3Mg_1S_2$	2694	(Mg_1S_2)	*3541*
$Ca_1Mg_1S_3$	3409	$Ca_2Mg_1S_3$	5150	$Ca_3Mg_1S_3$	4046	(Mg_1S_3)	*4202*
(Ca_1Mg_1)	*4820*	(Ca_2Mg_1)	*3606*	(Ca_3Mg_1)	*3355*	(Mg_1)	*3927*
$Ca_1Mg_2S_1$	4796	$Ca_2Mg_2S_1$	4690	$Ca_3Mg_2S_1$	2318	(Mg_2S_1)	*3935*
$Ca_1Mg_2S_2$	6570	$Ca_2Mg_2S_2$	4430	$Ca_3Mg_2S_2$	3618	(Mg_2S_2)	*4873*
$Ca_1Mg_2S_3$	7669	$Ca_2Mg_2S_3$	5751	$Ca_3Mg_2S_3$	4068	(Mg_2S_3)	*5829*
(Ca_1Mg_2)	*6345*	(Ca_2Mg_2)	*4957*	(Ca_3Mg_2)	*3355*	(Mg_2)	*4879*
$Ca_1Mg_3S_1$	6118	$Ca_2Mg_3S_1$	3194	$Ca_3Mg_3S_1$	4054	(Mg_3S_1)	*4455*
$Ca_1Mg_3S_2$	6131	$Ca_2Mg_3S_2$	4635	$Ca_3Mg_3S_2$	1846	(Mg_3S_2)	*4204*
$Ca_1Mg_3S_3$	4992	$Ca_2Mg_3S_3$	3466	$Ca_3Mg_3S_3$	4939	(Mg_3S_3)	*4466*
(Ca_1Mg_3)	*5747*	(Ca_2Mg_3)	*3765*	(Ca_3Mg_3)	*3613*	(Mg_3)	*4375*
(Ca_1)	*5637*	(Ca_2)	*4110*	(Ca_3)	*3434*	(ExP)	*4393*
(Ca_1S_1)	*5414*	(Ca_1S_2)	*6141*	(Ca_1S_3)	*5357*		
(Ca_2S_1)	*3781*	(Ca_2S_2)	*3758*	(Ca_2S_3)	*4789*		
(Ca_3S_1)	*3232*	(Ca_3S_2)	*2719*	(Ca_3S_3)	*4351*		
(S_1)	*4142*	(S_2)	*4206*	(S_3)	*4832*		

[1] Italics indicate means for levels of individual elements, e.g., Ca_1, or of element pairs, e.g., Ca_1Mg_1.

[2] Ca_1 = 160 mg. of calcium per litre; Mg_1 = 28 mg.; S_1 = 192 mg. The second and third levels of each element were respectively 1.5 and 2.0 times the first level.

[3] All totals corrected for block effect (incomplete block design).

Differences required for significances	5% level	1% level
Between totals	8,348	11,270
Between means for combinations of two elements	1,524	2,057
Between means for single elements	880	1,188

The number of progeny (eggs and active forms) 35 days after each plant was infested was the criterion of effect of the levels of the element supplied.

RESULTS

Within each replicate there was little variation in growth or colour of the plants save for treatment combinations 231 (2nd level of calcium, 3rd level of magnesium, 1st level of sulphur), and 311 (3rd level of calcium, 1st level of magnesium, 1st level of sulphur), which showed consistently poor plant growth. However, mite numbers were not affected adversely when plants received either of the above treatment combinations. The number of progeny decreased progressively as dosage of calcium was increased, the decreases being significant at the 1 per cent level between level 1 and 2 but not between levels 2 and 3 (Table 1). A decrease of 39 per cent between levels 1 and 3 was significant at the 1 per cent level. There were no significant differences in number of progeny between levels 1 and 3 when magnesium and sulphur were increased; but increases in magnesium between levels 1 and 2 resulted in increased number of progeny significant at the 5 per cent level.

An analysis of variance (Table 2) showed none of the interactions to be significant. When the variances were partitioned into linear and quadratic components (1; 8, pp. 42-48), only the linear effect of calcium was highly significant, i.e., increasing calcium resulted in a linear decrease in

TABLE 2.—ANALYSIS OF VARIANCE OF NUMBERS OF THE TWO-SPOTTED SPIDER MITE REARED ON NUTRIENT SOLUTIONS WITH VARIOUS LEVELS OF CALCIUM, MAGNESIUM, AND SULPHUR

Treatment combination	Linear and quadratic components	Degrees of freedom	Mean square[1]	F	F	
					5 per cent level	1 per cent level
Ca	L	1	8059686.0000	27.751**	4.05	7.21
	Q	1	469157.5432	1.6	4.05	7.21
Mg	L	1	475453.5000	1.6	4.05	7.21
	Q	1	899140.4876	3.1	4.05	7.21
S	L	1	971233.6666	3.3	4.05	7.21
	Q	1	228390.1799	—	—	—
CaMg	LXL	1	256880.0277	—	—	—
	Rest	3	309035.1801	1.1	2.81	4.24
CaS	LXL	1	172778.7777	—	—	—
	Rest	3	326905.1623	1.1	2.81	4.24
Mgs	LXL	1	9120.2500	—	—	—
	Rest	3	2183.4864	—	—	—
CaMgS	LXLXL	1	277512.5000	—	—	—
	Rest	7	431244.1764	1.5	2.22	3.05

[1] Tests of significance in this table are to be made against the original error variance, i.e., Mean Square for error, 290418.3731.
** Significant at the 1 per cent level.

number of progeny, over the three level dosages studied, of 27 per cent between levels 1 and 2, and 16 per cent between levels 2 and 3. Both the quadratic effect of magnesium and the linear effect of sulphur were significant at the 5.3 per cent level. Increasing magnesium resulted in a 24 per cent increase in number of progeny between levels 1 and 2 and a 12 per cent decrease between levels 2 and 3. Increasing sulphur increased the number of progeny linearly over the range of dosages studied by 1 per cent between levels 1 and 2 and 12 per cent between levels 2 and 3. It is interesting to note that the above negative linear response to calcium and positive linear response to sulphur by *T. telarius* are responses to elements that might be expected to have opposite effects on pH.

Total progeny per replicate was 28,234 for replicate I; 40,240 for replicate II; and 58,679 for replicate III. These totals differed significantly at the 1 per cent level. Totals for blocks within replicates did not show significant differences.

DISCUSSION AND CONCLUSIONS

The results show that increasing calcium fed to cucumber decreased the fecundity of *T. telarius*. This agrees with results of Wittwer and Haseman (7), who found thrips injury on spinach to be almost absent at high levels of calcium (40 milliequivalents per crock) and to be considerably reduced on low nitrogen groups when the calcium supply was increased; and also with those of Rodriguez (5), who found that the mean number of progeny per female of *T. telarius* decreased from 22 to 16 when calcium supply (20 to 500 p.p.m., Table 4) showed a slight increase in calcium absorbed (8.7 to 10.6 p.p.m.). The latter author found, however, that two of his experiments which showed a positive correlation between amount of magnesium and mite numbers showed a corresponding relationship for calcium. His nitrogen study showed absorbed calcium to be positively correlated with mite numbers.

The smaller total number of progeny in replicate I appears to be due in part to the lower fecundity of the deutonymphs used; these were not reared on cucumber for as long as deutonymphs used in the later replications. Populations of the mite from which the deutonymphs were taken were originally obtained from clover and transferred to cucumber. It seems possible that, over several generations, there may have been selected a strain better adapted to cucumber.

The total number of mites on all plants receiving the highest calcium level was approximately 60 per cent of that on plants fed the lowest amount of calcium (Table 1). However, the progeny totals for all treatments indicate that heavy infestations of the mite would have developed for all levels of the minerals had the experiment been continued beyond the 35-day period, and none of the mineral combinations would have given economic control of the pest. But the data do suggest that, if calcium were used at appropriate levels in greenhouse fertilizer, two-spotted spider mite populations on cucumber would be kept at lower densities.

The effects of various levels of calcium, magnesium, and sulphur on amount and edibility of fruit produced was not determined, the 35-day period of the experiment being too short for a test.

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FERTILITY OF BARLEY AUTOTETRAPLOIDS

I. FERTILITY IN SUCCESSIVE GENERATIONS OF FOUR AUTOTETRAPLOID BARLEY VARIETIES AND THE EFFECT OF SELECTION FOR FERTILITY IN THE O.A.C. 21 AUTOTETRAPLOID[1]

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[Received for publication July 16, 1958]

ABSTRACT

The differences in fertility of four colchicine-induced autotetraploid barley varieties (Brant, Montcalm, O.A.C. 21 and York) were determined and compared in four successive generations following the induction of tetraploidy. Despite a wide fertility range within each autotetraploid, the varieties tested varied considerably in their mean per cent fertility. Within each variety the mean per cent fertility remained relatively constant from generation to generation. The Montcalm tetraploid had the lowest mean fertility, fluctuating from generation to generation within a range of 6.0 to 10.1 per cent. The O.A.C. 21 tetraploid had the highest mean fertility, fluctuating within a range of 40.0 to 51.3 per cent.

Significant differences in fertility of the four autotetraploid varieties were interpreted as indicating that seed-setting ability may be genetically controlled and, therefore, hybridization and subsequent selection could be a promising method for increasing fertility.

Continuous selection for either high or low fertility from the C_1 to C_4 generation did not change the mean per cent fertility level in the O.A.C. 21 tetraploid.

INTRODUCTION

Colchicine has been widely used to produce autotetraploids in economic crop species. The induced autotetraploids very often have larger fruits or seeds, broader leaves and sturdier stems than the diploids. However, autotetraploidy is usually accompanied by partial sterility. This is a great disadvantage in those field crops where seed is the important end product.

Selection for the improvement of fertility in artificially induced autotetraploids[5] has been successful in rye, buckwheat, oil seed rape, red clover and alsike clover (1, 2, 3), and several varieties of tetraploid rye are in commercial production. By contrast, all attempts to improve the fertility of tetraploid barley sufficiently to warrant commercial production have failed.

Several authors (5, 6, 7, 10, 11) reported that fertility in tetraploid barley varies with the variety and environment. Müntzing (5) expressed the belief that the gap in fertility between diploids and tetraploids could be decreased. Ono (6, 7, 8) observed fertility for seven generations in 19 artificially induced tetraploid barley strains. In the two-rowed types, fertility progressively increased from 59.4 per cent in the C_1 to 86.1 per cent in C_7. In the six-rowed types, however, no increase in fertility was obtained. Ono expressed optimism that successive selection might increase fertility in certain tetraploid barley varieties.

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[5] Hereafter referred to as tetraploids. The term tetraploid will also be used in this paper for varieties and populations of barley which basically consist of plants with 28 chromosomes but could contain aneuploids with small deviations in chromosome number from that of the true tetraploid.

Müntzing (5) showed that the yield of hybrid tetraploids* was considerably higher than the yield of tetraploids obtained from standard varieties. Similar results were obtained by Rommel (10). Müntzing (5) postulated that it should be possible to select for fertility in the offspring of F_1 tetraploids in order to obtain highly fertile tetraploid lines.

The purpose of this study was to induce tetraploidy in several barley varieties, to compare fertility in subsequent generations of the induced autotetraploids, and to determine the effect of selection on fertility in the O.A.C. 21 tetraploid.

MATERIALS AND METHODS

In order to develop a method of colchicine treatment applicable to a wide range of barley varieties, preliminary work on the induction of tetraploidy in barley was carried out in 1954 at the Ontario Agricultural College. Three varieties, differing considerably in morphology and source of origin (O.A.C. 21, Hannchen and Jet), were used. A treatment schedule was developed that produced a relatively high average number of tetraploids (up to 10 per cent) in each of the three varieties tested. The schedule was as follows: Barley seeds were germinated in Petri dishes on moist filter paper at room temperature to the point where the coleoptiles (acrospires) started to emerge at the apical end of the kernels and the rootlets were approximately one-half to one inch long (36 to 48 hours). The germinating seeds were then placed in a shallow layer of 0.1 per cent aqueous colchicine solution for 3 hours. The treatment was prolonged if the acrospires were longer. After the treatment, the material was washed in tap-water and transplanted directly to soil in the greenhouse. This method was used to induce tetraploidy in the six-rowed varieties, O.A.C. 21, Montcalm, Brant and York. One hundred seeds of each variety were treated with colchicine. The tetraploid population of O.A.C. 21 obtained in the preliminary tests was added to the tetraploids obtained from this treatment.

The initial screening for tetraploidy was carried out in the C_1 generation. In the spring of 1955 the seed of one head from each of the C_0 plants, i.e. plants produced from colchicine-treated seed, was planted in a 5-foot row in the experimental field at the Ontario Agricultural College. The tetraploid plant rows, and rows containing both tetraploid and diploid plants, were readily distinguishable. The tetraploids were characterized by long kernels (especially laterals), incomplete emergence of the head, low fertility, and a low rate of tillering. The height of the plants seemed to be a reliable indicator under field conditions but was later found to be unreliable under greenhouse conditions. Each of the visually determined tetraploid plants was checked for chromosome number. For this purpose, two or three seeds of each plant were germinated in Petri dishes. The root tips were fixed in a combined stain-fixative (3:2:1) as described by Peters (9). After fixation, the root tips were boiled in a small glass vial of acetocarmine and subsequently squashed in a fresh drop of acetocarmine on a slide. By this method, up to 50 plants per day could be checked for tetraploidy.

* F_2 generation after hybridization and subsequent doubling

The cytological examination did not reveal a single diploid plant among the visually determined tetraploids, indicating the reliability of the visual checking method. This method was also found to be effective in eliminating the obviously diploid plants in the C_0 generation.

In the C_1 generation, spikes of the main tillers of tetraploid plants were harvested and used for fertility determinations. Per cent fertility was determined by the following procedure: The 15 spikelets attached to the 5 lower internodes of the spike were discarded and the fertility of the plant was recorded as the percentage of seed set in the next 30 spikelets. This method was used in all the tetraploid barley generations studied, as well as in the diploid controls. A pedigree system was adopted by which every plant in subsequent generations could be traced back to its C_1 parent.

The fertility in the C_2 generation of the four tetraploid barley varieties was determined in greenhouse-grown material when two to eight seeds from each C_1 plant were grown in a 7-inch pot at Winnipeg in May, 1956. Remnant seed of the C_1 generation of O.A.C. 21 tetraploids was grown in a greenhouse at Guelph for additional data on fertility.

The fertility in the C_3 generation of all four of the tetraploid barley varieties was determined in field-grown material at Guelph during summer of 1956. Each C_2 plant was represented by approximately 20 seeds planted in a 5-foot row.

The fertility in the C_4 generation of the four tetraploid varieties was determined in greenhouse-grown material at Winnipeg in January, 1957. For the tetraploid O.A.C. 21, 200 seeds from the most fertile plants in C_3 with high fertility pedigrees in C_2 and C_1, and 200 seeds from the most sterile C_3 plants having low fertility pedigrees in C_2 and C_1, were planted. For the York and Brant tetraploids, 40 seeds, picked at random, were planted in the C_4 generation. As the seed-set in Montcalm was very low every available seed was planted.

Three of the tetraploid varieties (excluding Montcalm) were tested for fertility under field conditions at Guelph in 1957. Approximately 200 seeds, taken at random, were space planted for each variety in this generation.

In all tetraploid generations, plants of the corresponding diploid varieties were included as checks. Since the diploids were almost completely fertile in all tests the data on fertility of the tetraploids required no correction.

In the C_4 generation, root tips of every plant grown were tested for exact chromosome number. For this purpose, each seed was planted separately in a 4-inch pot. Cold water pretreatment, as suggested by A. Mochizuki*, was used to shorten the chromosomes as follows: The excised root tips were placed in glass vials filled with tap-water and kept in a refrigerator at 2° C. for 24 hours. They were then fixed in Farmer's solution for at least 24 hours, stained with acetocarmine, boiled and squashed in acetocarmine as described for the C_1 material. No diploid plants were found in the C_4 generation.

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Four generations of the O.A.C. 21 tetraploid were compared for fertility under identical environmental conditions in a randomized block experiment conducted at the Ontario Agricultural College in 1957. In this experiment, a random sample of C_2, C_3 and C_4 generation seed was used, but the C_5 generation was represented by seed obtained from plants known to have 28 chromosomes in C_4. Plants were spaced 8 inches apart within rows and eight rod rows were used per plot in a four-replicate test.

For statistical comparisons, the results, obtained on a per cent basis, were transformed into the form $p = \sin^2\theta$.

EXPERIMENTAL RESULTS

The fertility means of the four tetraploid barley varieties in successive generations are presented in Table 1.

TABLE 1.—MEAN PER CENT FERTILITY OF FOUR TETRAPLOID BARLEY VARIETIES IN SUCCESSIVE GENERATIONS GROWN AT DIFFERENT LOCATIONS

Generation	Location	O.A.C. 21		Brant		York		Montcalm	
		No. of plants tested	Fertility in per cent	No. of plants tested	Fertility in per cent	No. of plants tested	Fertility in per cent	No. of plants tested	Fertility in per cent
C_1	Field Guelph	238	41.9	9	22.0	8	19.7	17	6.0
C_2	Greenhouse Winnipeg	704	40.0	17	42.4	20	38.7	6	7.3
C_2	Greenhouse Guelph	617	51.3	–	–	–	–	–	–
C_3	Field Guelph	3688	46.4	62	42.0	101	31.9	3	10.1
C_4	Greenhouse Winnipeg	212	49.5	25	47.0	21	38.5	2	6.3
C_5	Field Guelph	177	50.4	164	50.5	133	23.0	–	–

In the C_1 generation all of the varieties tested, except O.A.C. 21, had lower fertility than in later generations. As screening for tetraploidy was carried out in this generation, the competition with more vigorous diploid plants which were present may have been a partial cause for low fertility. The mean per cent fertility fluctuated within each variety from generation to generation with no apparent trend for increased or decreased fertility with advancing generations.

Environment appeared to affect the fertility of the four varieties tested. Except for the C_5 generation, which was grown under very favourable conditions, the generations grown in the greenhouse showed better fertility than those grown in the field. There was a highly significant difference in the fertility of O.A.C. 21 tetraploids grown in two different greenhouses, i.e. at Guelph and at Winnipeg.

Within each generation, the varieties differed considerably in mean per cent fertility. The "t" values for statistical comparison of the variety means are given in Table 2.

TABLE 2.—THE "T" VALUES FOR THE COMPARISON OF THE MEAN PER CENT FERTILITY OF FOUR TETRAPLOID BARLEY VARIETIES IN C_1 TO C_5 GENERATIONS. (CALCULATED USING TRANSFORMED DATA)

Varieties compared	Generation				
	C_1	C_2	C_3	C_4	C_5
O.A.C. 21—Brant	6.76**	0.37	1.40	0.40	0.03
O.A.C. 21—York	4.96**	0.10	7.25**	1.98*	16.60**
O.A.C. 21—Montcalm	20.15**	5.19**	5.28**	9.80**	–
Brant—York	0.38	0.25	2.88**	1.10	15.70**
Brant—Montcalm	4.03**	4.23**	4.44**	6.56**	–
York—Montcalm	4.14**	2.62*	3.38**	5.79**	–

*, ** Significant at 5% and 1% levels, respectively

Except in the C_1, wherein O.A.C. 21 was significantly more fertile than all the other three varieties, there were no significant differences in fertility between O.A.C. 21 and Brant. These two tetraploids consistently had the highest average fertility. In all generations, the Montcalm tetraploid was significantly lower in fertility than the other three tetraploids. This low fertility, coupled with consistently low germinability, made it difficult to obtain satisfactory stands of the Montcalm tetraploid under ordinary growing conditions. In order to maintain the Montcalm tetraploid, it was grown under different environmental conditions in 1957 and, therefore, the C_5 data of this variety were not included in the comparison.

The fertility data of four generations of O.A.C. 21 tetraploid, grown at the same time under identical environmental conditions, are presented in Table 3.

TABLE 3.—MEAN PER CENT FERTILITY OF FOUR GENERATIONS OF O.A.C. 21 TETRAPLOID BARLEY GROWN IN 1957 AT GUELPH

Generation	Fertility in per cent				
	Rep. I	Rep. II	Rep. III	Rep. IV	Mean
C_2	20.3	39.5	40.2	27.6	31.9
C_3	27.9	31.2	41.4	38.0	34.6
C_4	31.6	34.4	39.9	44.8	37.7
C_5	42.4	47.1	40.2	42.9	43.2

An analysis of variance, using transformed data, indicated no significant differences in mean per cent fertility at the 5 per cent level ($F = 2.84$). However, there appears to be a trend for increased fertility with advancing generations. A test for linearity indicated that this trend was linear ($F = 8.3^*$) with no quadratic or cubic effects. The regression coefficient ($b = 2.26 \pm 1.24$), however, indicated that the increase in fertility from generation to generation was very small.

*Significant at the 5% level

The high inter-replicate fluctuation in per cent fertility, particularly in each of the C_2, C_3 and C_4 generations, is indicative of the sensitivity of the tetraploids to environmental conditions. The greater uniformity of fertility in the C_5 generation, although probably due to chance, could be a result in part of the elimination of aneuploid plants in the parent C_4 generation.

In order to find the distribution of fertility within each tetraploid population, ten different classes were established at the outset and the frequency of plants in each class was recorded. Class values ranged from 6.7 per cent to 96.7 per cent and the class intervals were 10 per cent. The data obtained indicated that the fertility varied greatly from plant to plant within each variety. In the varieties where the populations were fairly large, some highly fertile plants (95 per cent) were found. In the C_3 generation of O.A.C. 21, where the population was large the frequency distribution for fertility approached a normal curve.

A possible explanation for the wide range in fertility in each generation of each of the tetraploids is that the progeny of plants with low fertility would likewise be low in fertility. Similarly, plants with high fertility would produce highly fertile progeny. If this hypothesis were true, it would mean that selection for fertility is possible. The O.A.C. 21 data were arranged to test this hypothesis. The offspring of plants from each of the ten fertility classes established in the C_1 generation were rated for fertility in the two C_2 populations. Likewise the offspring from plants of each of the fertility classes established in C_2 were rated for fertility in the C_3 generation. From the data obtained, it could be seen that, in general, the plants with low fertility parents did not differ in fertility from plants which had highly fertile parents. In the C_3 generation, in which each fertility class was well represented, even the offspring of the 6.7 per cent fertility class did not differ in fertility from the offspring of the 86.7 per cent fertility class. For graphical presentation the fertility data in the two C_2 populations and in the C_3 generation were arranged in two groups. All plants from parents with fertility below 40 per cent were included in one group, and plants from parents with fertility above 40 per cent were included in the other group.

The C_4 generation data were also arranged in two groups. One group represented only plants with pedigrees that never exceeded the 40 per cent fertility level in any one of the previous generations. The other group included plants having pedigrees with fertility higher than 40 per cent in all the previous generations. The fertility data of the two groups in each generation are represented by histograms in Figure 1.

From the histograms, it can be seen that selection of plants with high or low fertility in any of the three generations did not change the fertility distribution in the subsequent generation.

The fertility means of the two groups in each generation are presented in Table 4.

The "t" values indicate that in the C_2 generation grown at Winnipeg, and in the C_4 generation, the differences between the fertility means of the two groups were not significant. In the C_2 generation grown at Guelph, the offspring of the low fertility class had better mean fertility than that of the high fertility C_1 class.

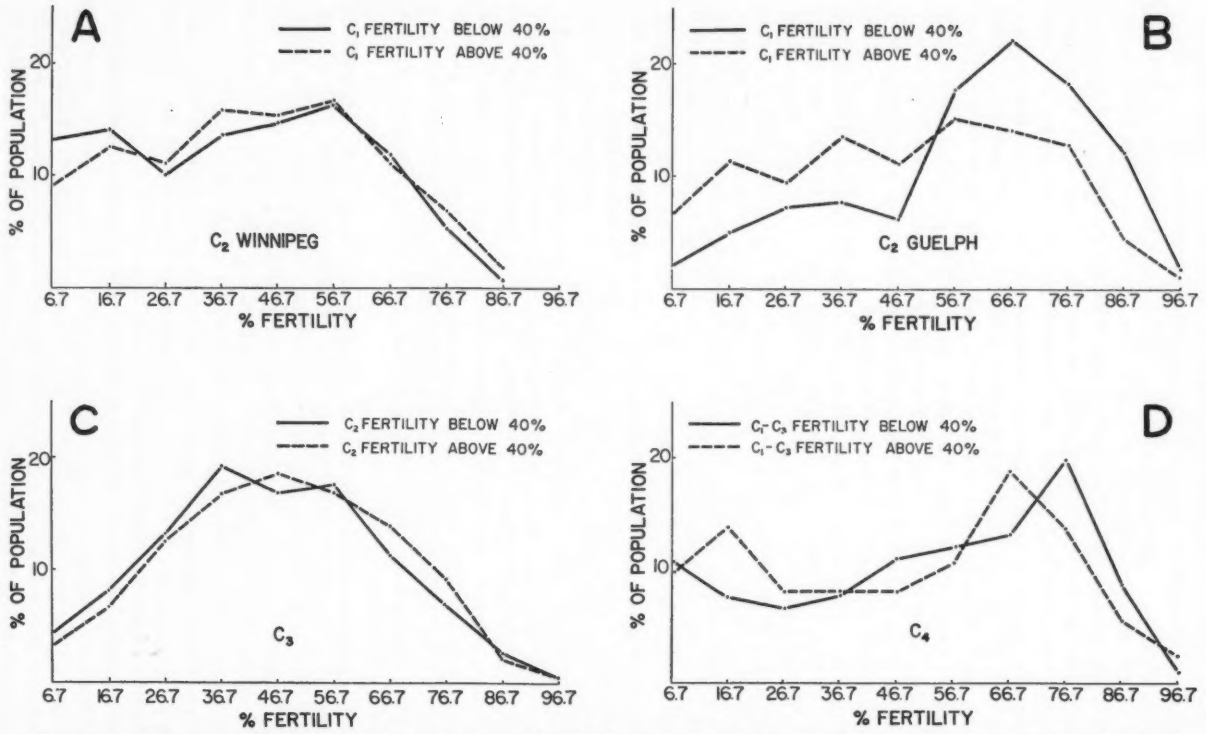

FIGURE 1. Frequency distribution of plants at various fertility levels in C_2 (A—grown at Winnipeg, B—grown at Guelph); C_3 (C) and C_4 (D) generations of tetraploid O.A.C. 21 barley derived from two fertility classes in previous generations.

TABLE 4.—MEAN PER CENT FERTILITY OF PLANTS IN THE C_2, C_3 AND C_4 GENERATIONS DERIVED FROM TWO FERTILITY CLASSES (BELOW AND ABOVE 40 PER CENT) OF PREVIOUS GENERATIONS OF TETRAPLOID O.A.C. 21 BARLEY

Generation	Fertility class of the previous generations	Number of plants	Fertility in per cent	"t"
C_2 Winnipeg	C_1 below 40% C_1 above 40%	286 418	38.2 41.2	1.68
C_2 Guelph	C_1 below 40% C_1 above 40%	181 436	60.0 47.6	5.76**
C_3	C_2 below 40% C_2 above 40%	1378 2488	44.7 47.3	3.45**
C_4	C_1 to C_3 below 40% C_1 to C_3 above 40%	90 122	51.4 48.1	0.79

** Significant at the 1% level

In the C_3 generation, the mean fertility in the first group was only 2.6 per cent lower than that in the second group. The "t" test applied to the means indicated that this difference was significant. The histogram shown in Figure 1-C clearly indicates, however, that there was very little difference in fertility between the two groups in the C_3 generation, and obviously there would be no advantage gained by selecting fertile plants from the higher fertility pedigree group.

The C_4 generation test was repeated on a larger scale, using four replications (approximately 120 plants per replication) at Guelph in 1957. The mean per cent fertility in this test for the low and high fertility pedigree groups was 36.9 and 38.5 respectively. An analysis of variance of transformed data indicated that this difference in fertility was not significant ($F = 1.01$).

The statistical data for the four generations of the tetraploid O.A.C. 21 barley support the conclusions drawn from the histogram, i.e. that selection for either low or high fertility was ineffective.

DISCUSSION AND CONCLUSIONS

One of the observations in this study was that each autotetraploid barley had a specific mean fertility which could be influenced to a certain extent by the environment. This is in agreement with several reports (5, 6, 7, 10, 11) which indicate that the fertility in autotetraploid barley varies with the variety and environment. Although the four tetraploids varied considerably in mean per cent fertility, the highest mean was only 51.3 per cent. It is highly unlikely that the entire range in mean fertility would have been included in so small a sample. If tetraploidy were induced in a large number of diploid varieties, it is probable that more fertile tetraploids would be obtained than herein reported.

Müntzing (4) stated that, because of selection, autotetraploids occurring in nature are more fertile than artificially induced ones. Ono (8) indicated that it should be possible to breed a tetraploid barley variety with high fertility through successive selection. The results obtained in this study (Table 1) showed very small change in fertility in the autotetraploid barley varieties tested from C_1 to C_5. There were indications (Table 3) that natural selection for increased fertility in tetraploid barley may be rather slow and thus could not be readily detected in the four generations studied. On the basis of the data presented in Figure 1, selection for fertility would not be recommended as a method for increasing the fertility in subsequent generations. However, the null response to selection obtained in O.A.C. 21 barley may not be representative of the response to selection in other tetraploid barley varieties and, therefore, selection as a tool for improving fertility should not be entirely ruled out.

The diploid barley varieties chosen for this study were highly homozygous. Therefore, the autotetraploids obtained by chromosome doubling would also be homozygous. The possibility of selecting for higher fertility in such populations could be based only on the assumption that the plants in early generations after doubling would have an abnormal chromosome complement which can be stabilized later. The data from this study, however, indicate that the fertility differences between the varieties remained relatively constant from generation to generation. This indicates that the seed-setting ability in autotetraploid barleys may be genetically controlled.

Müntzing (5) postulated that it should be possible to obtain a highly fertile autotetraploid barley variety by hybridization. Since large differences were found to exist among tetraploids of the four varieties used in the present study, it is probable that a greater range in fertility would be found among induced tetraploids of heterogeneous material created by hybridization.

ACKNOWLEDGEMENTS

The writers wish to express appreciation to the Brewing and Malting Barley Research Institute, Winnipeg, Man., for the financial support which made the project possible.

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EFFECTS OF ARTIFICIAL DEFOLIATION ON SUNFLOWERS[1]

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[Received for publication April 8, 1958]

ABSTRACT

Sunflowers were defoliated artificially to various degrees at three stages of growth to simulate the effects of rust and other foliage diseases. Complete defoliation at the flowering stage was most injurious. It reduced seed yield by 88 to 93 per cent in all five years of the experiment. It also reduced plant height at maturity in one of two years in which heights were recorded, and it reduced weight of 200 seeds, and oil content and protein content of the seed. Removal of 50 per cent of each leaf at flowering reduced seed yield by 22 to 30 per cent, and reduced plant height, but did not cause other statistically significant reductions. Removal of all leaves on the upper half of the stem reduced yield and 200-seed weight significantly.

Complete defoliation of seedlings reduced seed yields significantly in two years and reduced plant height in one year. Complete defoliation of maturing plants reduced seed yield significantly in one year. Partial defoliation (50 per cent and 25 per cent) of seedlings and maturing plants produced no significant effects.

The results of artificial defoliation should be useful in evaluating damage caused by insects and by hail as well as the effects of foliage diseases.

INTRODUCTION

Leaf injury to sunflowers caused by rust (*Puccinia helianthi* Schw.), leaf spot (*Septoria helianthi* Ell. & Kellerm.), and other diseases was noted in Manitoba before sunflowers were grown commercially as an oil-seed crop (1). Commercial production of sunflowers began in Manitoba in 1943 (5) and not long after growers commenced to complain of disease problems. When the author began to study sunflower diseases in 1947, no quantitative data on the effects of foliage diseases were available. Chester (2) had pointed out that yield reduction of wheat due to artificial defoliation was comparable to that caused by leaf rust; when there was a difference, the disease reduced yields more than an equal amount of artificial defoliation. It was decided, therefore, to use mechanical defoliation as a measure of the effects of rust and other leaf diseases on the yield of sunflowers.

Direct studies on the quantitative effects of rust on sunflowers were begun in 1949. In that same year, leaf injuries caused by hail and by insect feeding, similar in extent and pattern to the mechanical defoliation used in these experiments, were observed in farm fields. As it became apparent that the results of defoliation tests might have wider applicability than was originally expected, the experiments were continued. A preliminary report on results for two years was presented in 1950 (6). The present paper records the results of experiments made during the five years 1948 to 1952 inclusive.

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MATERIALS AND METHODS

All the experiments were made in field plots. The plots were sown at Winnipeg in each year except 1950, when a flood made it necessary to move all field experiments to an area at Headingly, 12 miles west of Winnipeg. The hybrid variety Advance was used from 1948 to 1951. In 1952 Sunrise, the pollen parent of Advance, was sown.

All the experiments were designed as randomized blocks, with all treatments occurring once in each replicate. The rows were spaced 36 inches apart, and plants were thinned to approximately 6 inches apart in the rows shortly after emergence. Variations in details of the experimental design from year to year are given in Table 1.

The plants were defoliated at three stages of development, seedling, flowering, and maturing, in each year except 1952, when they were defoliated only at the flowering stage. The intervals from seeding to the respective defoliations and harvest in each year are recorded in Table 2.

Farmers usually delay harvesting until frosts have killed the sunflowers, so that they dry rapidly. As this practice was also followed in harvesting the plots, the differences in number of days from seeding to harvest in the various years indicate not differences in time to mature the crop, but in the dates of killing frosts.

The method and degree of defoliation were not the same in all years. The treatments in 1948 were 100 per cent and 50 per cent defoliation. In the 100 per cent treatment, all the expanded leaves were cut off with scissors, leaving only two partly expanded leaves at the growing point. In the 50 per cent treatment, half of each leaf was cut off longitudinally beside the midrib. A third treatment, 25 per cent defoliation, was added in 1949, 1950, and 1951. It consisted of removing the apical quarter of each leaf by making two cuts with scissors, the first longitudinal, extending half-way from the apex to the base along the midrib, and the second from

TABLE 1.—DETAILS OF EXPERIMENTAL DESIGN

Year	Number of replicates	Rows per plot	Length of rows in feet
1948	4	1	5
1949	5	2	5
1950	6	1	12
1951	6	1	10
1952	4	2	10

TABLE 2.—DATES, AND DAYS AFTER SOWING, OF DEFOLIATION AND HARVEST

Year	Date sown	Seedling stage		Flowering stage			Maturing stage		Harvest	
		Date	Days after sowing	Date	Days after sowing	Stage	Date	Days after sowing	Date	Days after sowing
1948	May 26	July 9	44	July 28	63	Bud	Sept. 1	98	Sept. 20	117
1949	May 14	June 30	47	Aug. 3	81	Bloom	Sept. 1	110	Oct. 5	144
1950	June 6	July 23	48	Aug. 22	78	Bloom	Sept. 19	106	Oct. 13	130
1951	May 23	July 16	54	Aug. 20	89	Bloom	Sept. 6	106	Sept. 28	128
1952	May 13	.....	.....	Aug. 14	93	Past bloom	.....	.....	Sept. 22	132

the margin to the midrib to join the first. The three degrees of defoliation are illustrated in Figure 1. In 1952, four degrees of defoliation were used. The 100 per cent and 50 per cent treatments of previous years were repeated. In addition, two different ways for removing 50 per cent of the leaf surface were employed. In one, all the leaves were cut from the upper half of the stem; in the other, all the leaves were cut from the lower half of the stem.

The experiments were planned primarily to determine the effect of defoliation on seed yield. It proved necessary to bag individual heads as soon as possible after they finished flowering to prevent the seed being eaten by birds. Other data that were recorded included plant height at harvest in 1948, and at three stages of growth in 1951; and weight of 200 seeds, and oil and protein content of the seeds, in 1952.

It was difficult to obtain yield data in 1950. As the plots at Headingly were not readily accessible, the heads were not bagged soon enough after flowering, and all the seed was eaten by birds. An estimate of yield was made by tracing on clear cellophane the outline of each head, the zone of sterile seed, and the zone of seed removed by birds and, therefore, presumably fertile. The actual area of each zone was determined with a planimeter. The areas bearing fertile seed per plant and per plot were used instead of actual seed yield to assess the effects of the various treatments. The validity of this procedure was tested by making similar tracings and planimeter measurements on 25 undamaged heads from a farm field, threshing each head individually, and comparing the fertile seed areas with seed yield data. The coefficient of correlation between seed bearing area and seed yield was 0.95.

In no year was it possible to harvest all the heads from all plots. Some of the plants were broken over by wind or rain before harvest, others were killed by root rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. Seed yields, and in 1950 fertile seed bearing area, were therefore calculated on the individual plant basis rather than as yield per plot. All data were evaluated by analysis of variance.

EXPERIMENTAL RESULTS

Seed yields were determined as grams per plant and were then calculated as pounds of seed per acre, assuming a perfect stand of plants 6 inches apart in rows spaced 36 inches apart. The data for all treatments in all years are shown in Table 3.

The calculated yields of seed per acre for 1948 and 1949 are very much higher than those obtained in farm fields. They are also higher than the values obtained from small experimental plots in which all heads are harvested and threshed to give actual plot yields. The data for 1950 are based on seed-bearing area of the heads rather than on seed yield. The values for 1951 and 1952 are within the range of good farm and plot yields. In order to make comparisons between results for the various years easier, all the data were converted to a percentage of the values for the undefoliated check plots in the respective years. The percentage data are presented graphically in Figure 2.

FIGURE 1. Sunflowers showing three degrees of defoliation at the flowering stage: *A*, 100 per cent; *B*, 50 per cent; and *C*, 25 per cent.

TABLE 3.—EFFECT OF DEFOLIATION ON SEED YIELD OF SUNFLOWER PLANTS

Plant stage and degree of defoliation	Yield in pounds of seed per acre				
	1948	1949	1950[1]	1951	1952
Seedling					
100%	3889	4302	88.1	896	—
50%	5457	4366	72.5	1242	—
25%	—	4814	86.5	1274	—
Flowering					
100%	392	371	4.0	122	269
50% (midrib)	4032	3547	79.1	973	1671
50% (upper)	—	—	—	—	1069
50% (lower)	—	—	—	—	1921
25%	—	4725	73.7	1044	—
Maturing					
100%	4296	3816	73.7	1012	—
50%	5269	4277	84.2	1159	—
25%	—	4494	78.1	1376	—
Undefoliated check	5788	4725	82.8	1255	2234
L.S.D. 5%	1694	648	19.1	270	300
L.S.D. 1%	2562	870	25.5	360	421
L.S.D.[2] 5%	Not significant	713	7.9	303	328
L.S.D.[2] 1%		964	10.6	406	471

[1] Area bearing fertile seed, in square centimetres
[2] Data from plots completely defoliated at flowering omitted; least significant differences for revised data

The effect on yield of complete defoliation at the flowering stage is of a different order than the effects of other treatments. The data were therefore re-analysed, omitting values for plots completely defoliated at flowering, in order to assess with greater precision the differences resulting from the other treatments.

When the least significant differences derived from the recalculated data were used, fewer of the differences due to the respective treatments proved significant. None of the treatments (other than complete defoliation at the flowering stage) caused significant reduction in yield in 1948. The reduction caused by 50 per cent defoliation at flowering in 1949 was highly significant, and by 100 per cent defoliation of maturing plants was significant. Only complete defoliation of seedlings reduced yield significantly in 1951. Removal of all leaves on the upper half of the stem, and 50 per cent defoliation of all leaves along the mid-rib, both caused highly significant yield reduction in 1952. More differences in the data for 1950 proved significant when the values for 100 per cent defoliation at the flowering stage were omitted, than when those values were included. The area bearing fertile seed was reduced significantly by 50 per cent defoliation in the seedling stage, by 25 per cent defoliation in the flowering stage, and by 100 per cent defoliation of maturing plants. As 50 per cent defoliation of seedlings was apparently more injurious in 1950 than 100 per cent defoliation, and 25 per cent defoliation in the flowering stage was more injurious than 50 per cent, the data for fertile seed area in 1950 do not agree with those for actual seed yields obtained in the other years of these experiments.

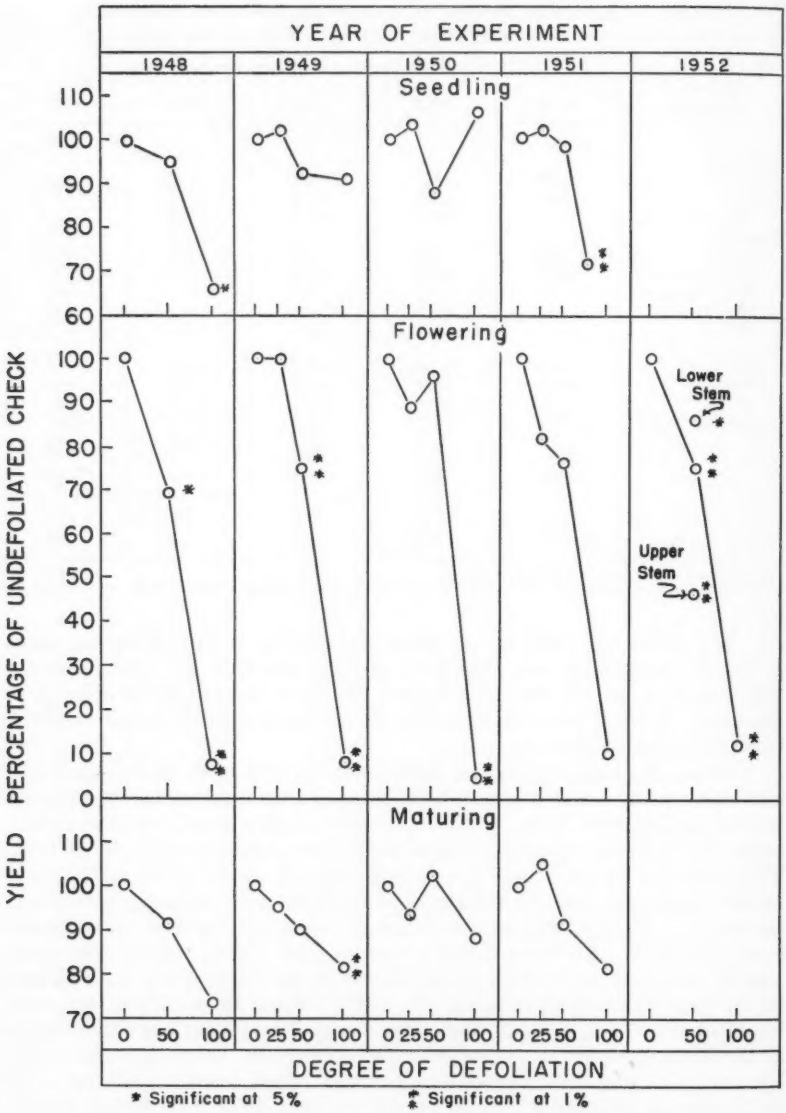

FIGURE 2. Effect of defoliation at various stages on yield of sunflowers.

Plant heights were recorded at harvest time in 1948, and at three stages in 1951. The height in inches of plants at harvest time in 1948, and 10 days before harvest in 1951, are given in Table 4.

TABLE 4.—EFFECT OF DEFOLIATION ON HEIGHT OF SUNFLOWER PLANTS

Plant stage and degree of defoliation	Height of stem in inches, not including bent-over portion	
	1948	1951
Seedling[1]		
100%	41	47
50%	53	49
25%	—	49
Flowering[2]		
100%	44	52
50%	46	51
25%	—	50
Maturing		
100%	50	49
50%	50	50
25%	—	50
Undefoliated check	53	48
L.S.D. 5%	6.92	Not significant
L.S.D. 1%	9.48	

[1] Plants defoliated in the seedling stage in 1948 were about 6 to 15 in. tall; in 1951 they were 26 to 32 in. tall when defoliated.

[2] Plants defoliated in the flowering stage in 1948 were actually in bud and 30 to 36 in. tall; in 1951 they were in flower, and from 52 to 56 in. tall.

TABLE 5.—EFFECT OF DEFOLIATION OF SUNFLOWERS ON WEIGHT OF 200 SEEDS, OIL CONTENT AND PROTEIN CONTENT OF THE SEED, AND YIELD OF OIL AND PROTEIN PER ACRE

Degree of defoliation	Grams per 200 seeds	Oil per cent	Protein per cent	Oil pounds per acre	Protein pounds per acre
100%	4.88	19.50	27.05	52	59
50% (upper)	7.15	30.20	33.50	323	250
50% (midrib)	8.89	32.25	31.68	539	359
50% (lower)	8.88	31.35	30.22	602	398
Undefoliated check	9.69	32.28	31.50	721	477
L.S.D. 5%	0.95	2.31	2.75		
L.S.D. 1%	1.34	3.25	3.86		

The weight per 200 seeds was determined for samples of seed from every plot in 1952. Other samples were analysed for oil content and protein content at the Grain Research Laboratory of the Board of Grain Commissioners in Winnipeg. The oil and protein percentages were used to calculate the yield in pounds of oil and pounds of protein per acre from plants subjected to the various defoliation treatments. The data are presented in Table 5.

TABLE 6.—CORRELATION COEFFICIENTS FOR ALL INTERRELATIONSHIPS BETWEEN YIELD, SEED WEIGHT, OIL CONTENT, AND PROTEIN CONTENT

	Yield		Seed weight		Oil content	
	(1)	(2)	(1)	(2)	(1)	(2)
Seed weight	0.95**	0.92**				
Oil content	0.74**	0.29	0.84**	0.31		
Protein content	0.25	−0.50*	0.36	−0.57*	0.72**	0.23

[1] All data included
[2] Data from completely defoliated plots omitted
* Significant. Required for significance at 5% level for columns (1), 0.44 and for columns (2), 0.497
** Highly significant. Required for significance at 1% level for columns (1), 0.56, and for columns (2), 0.623

Complete defoliation caused highly significant reduction of seed weight, and oil content and protein content of the seed. Removal of all leaves on the upper half of the plant also caused highly significant reduction of seed weight, but did not affect either oil or protein content significantly. The effects of the respective treatments on yields of oil and of protein per acre were very striking. As these data were calculated from mean values of oil and protein percentage, and mean yields of seed per acre, they were not analysed statistically.

Omission of the values for complete defoliation at the flowering stage did not alter the results of statistical analyses of 200-seed weight, oil content, or protein content.

The simple correlation coefficients were calculated for all interrelationships between yield, weight of 200 seeds, oil content, and protein content of the seed.

The effect of complete defoliation at flowering was so much greater than that of the other treatments that the correlation coefficients were recalculated, omitting values for complete defoliation, to see what relationships existed among the remaining data. The recalculated coefficients are given in Table 6.

The only correlation not altered appreciably by the omission of values from completely defoliated plots was that between yield and seed weight. The other relationships indicated by highly significant correlation coefficients in Table 6 were different either in degree or in kind when the most drastic treatment was omitted.

DISCUSSION AND CONCLUSIONS

Complete defoliation of sunflower plants in the flowering stage is a drastic treatment that almost destroys their capacity to produce seed. This effect is predictable, since the seed is dependent on the production of food materials by the leaves. Removal of 50 per cent of the leaf surface at the flowering stage also reduced seed yield significantly, from 22 to 30 per cent below that of the undefoliated check. Removal of 25 per cent of the leaf surface at flowering did not reduce seed yield in 1949 and caused a non-significant reduction of 17 per cent in 1951.

The data for 1952 show that not all leaves or parts of leaves are of equal importance to the plants. When all leaves were removed from the upper half of the stem, the reduction in yield was about 52 per cent. When the 50 per cent defoliation was made by removing half of each leaf longitudinally along the midrib, the loss was less, about 25 per cent. This effect was greater than the 14 per cent reduction which resulted when all the leaves were removed from the lower half of the stem.

The importance of the upper and lower leaves respectively in determining seed yield is a function of their physiological activity and efficiency. The basal leaves are approaching senescence at flowering time, whereas the upper leaves are relatively young and photosynthesizing actively. Removal of a given proportion of the upper leaves, therefore, has a much greater effect on the plant than removal of the same proportion of the less efficient lower leaves. Removing part of each leaf on the plant could be expected to have the observed intermediate effect.

Chester (3) states that the full complement of leaves functions at a relatively low efficiency. He uses the data of others to prove that the first leaves lost are dispensable, their removal causing less damage to the plant than further equal increments of defoliation. As more leaves are lost, those remaining function more and more efficiently and their loss is more detrimental to the plant than that of the first, less efficient leaves. It is possible that this relationship may apply also to the loss of the successive portions of individual sunflower leaves. The data from the experiments reported here are not sufficiently extensive and are too variable to justify such a generalization, although some of the results shown in Figure 2 seem to support it.

Defoliation of sunflowers in the seedling stage affected yield differently in different years. Complete defoliation caused a significant reduction in yield in 1948 and a highly significant reduction in 1951, but only a slight reduction in 1949. Removal of 50 per cent or 25 per cent of the seedling leaves had no significant effect on yield. Apparently in most years seedlings can recover from all but complete defoliation, growing new leaves in time to produce more or less normal quantities of seed.

Complete defoliation of maturing plants reduced seed yield significantly only in 1949. In that year, as in 1948 and in 1951, 50 per cent defoliation caused an appreciable, although not significant, yield reduction. The leaves of maturing plants have a fairly short period of usefulness remaining; their loss could be expected to have a relatively minor effect on yield. The actual percentage losses observed, although not significant in most cases, were sufficiently great to indicate that even partial defoliation of maturing plants has an adverse effect on yield.

The time of defoliation affects the results with corn, barley, oats, sorghums, onions, flax, and soybeans, just as it does for sunflowers. Chester (3) states that, if these plants are defoliated at various stages from early development to maturity, the loss in yield is greatest when they are defoliated in mid-season and progressively less with earlier or later defoliation. His explanation of this mid-season effect is that "at this critical stage in development the foliage has not yet served its photosynthetic function,

yet it is too late for the plant to develop a new set of leaves to compensate for those lost. With defoliation progessively earlier than this, the plant has more time to replace the lost foliage, which then is able to function fairly well, while with defoliation progressively later than the critical period, the leaves have served their purpose to an increasing extent, and to the same extent are dispensable."

The loss of varying proportions of leaf surface at various stages of growth may affect various processes differently than it does yield. Defoliation sometimes has little effect on one type of organ or may even stimulate its development, but at the expense of other organs and of the growth of the plant as a whole. An example of this is an increase in the yield of leaves of soybeans, but a reduction in seed production, as the result of moderate defoliation (3). An indication of this differential effect was observed in the sunflower experiments. Complete defoliation of seedlings in 1948 reduced both seed yield and plant height at maturity. Partial defoliation caused a non-significant reduction in seed yield but had no effect on plant height. Defoliation had no effect on plant height in 1951, although complete defoliation of seedlings caused highly significant reduction in seed yield.

The difference in effects of seedling defoliation in 1948 and 1951 on subsequent plant height reflects the difference in development of plants in the two years. The "seedlings" were defoliated in 1948, 44 days after sowing, and were from 6 to 15 inches tall; the "seedlings" in 1951 were defoliated 54 days after sowing, and were 26 to 32 inches tall. An even greater difference in development at the flowering stage in the two years accounts for the difference in effect of defoliation at this stage on plant height. Defoliation at "flowering" in 1948 was done 63 days after sowing, when the plants were in bud; in 1951 it was done 89 days after sowing, when the plants were in full bloom. In 1948 the plants were 30 to 36 inches tall when defoliated; in 1951 they were 52 to 56 inches tall. Sunflowers have usually attained almost their full height by the time they are in full bloom. Obviously, therefore, defoliation or similar injury of plants in bloom can have relatively little effect on their final height, irrespective of its effect on yield.

The full complement of florets is differentiated before the plants start to flower. The very great reduction in yield caused by defoliation at flowering is due, therefore, either to the failure of the florets to develop, or to the inability of the plant to fill the seeds after the florets are fertilized.

The effect of defoliation on the filling of seed is shown by the 200-seed weights. Seed weight was drastically reduced by complete defoliation at flowering, and was highly significantly reduced by removal of all leaves from the upper half of the stem. Longitudinal excision of all leaves at the midrib, and removal of all leaves from the lower half of the stem, both caused an appreciable but not quite significant reduction in seed weight. The extremely high correlation for variation of seed yield with seed weight shows fairly conclusively that yield was affected by reduction of the weight, or degree of filling of seeds, rather than by reduction of the number of seeds produced. Additional evidence is provided by the effect of defoliation on the area bearing fertile seed. Fifty per cent defoliation of plants

in the flowering stage reduced seed yields from 22 to 30 per cent in each year in which seed was harvested. In 1950, this treatment reduced fertile seed area only 4 per cent, indicating that the yield reduction in other years was due to reduction in weight, and not in number of fertile seeds. This additional evidence is of doubtful value, however, because of the irregularity of the 1950 results; 25 per cent defoliation reduced fertile seed area by 11 per cent.

The data for the effects of defoliation on oil and protein content of the seed are for one year only. In that year, complete defoliation reduced both oil content and protein content of the seed very strikingly; partial defoliation had no significant effect. As the yield of seed was reduced by partial defoliation, however, the total production of oil and of protein was reduced by all treatments, as indicated by the calculated yields of oil and of protein per acre.

Chester (3) quotes results showing that, as the degree of defoliation increases, the oil content of soybeans and the protein content of wheat are reduced. This linear relationship apparently does not hold for sunflowers. When the values for completely defoliated plants were omitted, the correlation coefficients showed that factors reducing seed yield and seed weight had no significant effect on the oil content, and significantly increased protein content of the seed. These results run counter to the general concept that plump seed tends to have higher oil content than light seed.

Peturson, Newton, and Whiteside (4) found that under certain conditions wheat leaf rust infection reduced the protein percentage of wheat, and under other conditions increased it. The factors that affected protein assimilation, as influenced by leaf rust, were not determined. The sunflower data are too limited to generalize but they show that at least under certain conditions partial defoliation may increase protein content of the seed.

The defoliation experiments reported in this paper were designed to provide a measure of the effects of leaf diseases, particularly rust, on the yield of sunflowers. The results of the defoliation studies should be directly applicable in assessing the damage caused to sunflowers at various stages of growth by hail and by the feeding of insects. The initiation of rust experiments in 1949 made it possible to measure the effects of rust directly, instead of applying the data from defoliation tests. A preliminary report on the effects of rust has been presented (7). The relationship between the effects of mechanical defoliation and the effects of rust infection will be discussed in the more detailed report on the rust studies which will appear in a later paper.

ACKNOWLEDGEMENTS

The author is grateful to E. D. Putt, formerly with Co-operative Vegetable Oils, Ltd., Altona, Man., for supplying the undamaged sunflower heads used to determine relationship between fertile seed area and seed yield in 1950; and to D. K. Cunningham, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Man., who analysed seed samples for oil and protein content in 1952.

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A STUDY OF THE EFFECTS OF TEMPERATURE AND OTHER FACTORS UPON THE GERMINATION OF VEGETABLE CROPS

II. PEAS[1]

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[Received for publication April 29, 1957]

ABSTRACT

A series of trials was conducted at two periods, spaced a year apart, to study the effect of temperature and of the duration of exposure upon the germination of three pea varieties, Lincoln, AA-15 and Thomas Laxton, without the complicating effects of soil-borne organisms. The results indicate that the germination of peas, when sown in vermiculite and held in controlled temperature chambers, was significantly reduced as the temperature was lowered from 40° to 35° F. The duration of exposure had little effect upon the over-all mean, although differences were significant in the first trial period. Varietal behaviour differed as a result of temperature and of duration of exposure. It is concluded that peas will tolerate exposure to temperatures as low as 40° F. for considerable periods of time, providing steps are taken to protect the seed against pathogenic organisms.

INTRODUCTION

Peas, for canning and freezing, constitute one of the major processing crops in the irrigated areas of southern Alberta. It is expected that the acreage producing peas will increase with the expansion of irrigation and the development of frozen-food plants.

"The pea is a cool season crop"—Jones and Rosa (4). "The pea thrives best under relatively cool weather"—Thompson (8). "Peas do well in cool moist weather and will germinate and make slow but healthy and vigorous growth in lower temperatures than most garden vegetables" —Bailey (1). These quotations denote the consensus regarding the temperature requirements of peas which could be expected to germinate satisfactorily under conditions of low soil temperature. However, stands of early-seeded peas are often poor, apparently as a result of the effects of the cold soil.

Although Kotowski (5) obtained good germination of peas, at temperatures as low as 39°F., he found that the velocity of germination increased as temperatures increased. He found, further, that all seed germinated at 65°F., but that seedling emergence fell off sharply at higher temperatures and more slowly at lower temperatures. These observations indicated that the optimum germinating temperature for peas was approximately 65°F., which is in close agreement with U.S. Department of Agriculture standards for germination tests (6).

In reviewing literature dealing with germination of peas, Jones and Rosa (4) noted that soaking pea seed in water was injurious, and that this injury was more marked at low temperatures (41° to 50°F.) than at medium soaking temperatures (59° to 68°F.). Increased injury was also

[1] Contribution No. 899 from the Horticulture Division, Experimental Farms Service, Canada Department of Agriculture.

reported at higher temperatures. In explanation, it was stated that injury at low temperatures probably was due to leaching out of essential food reserves and enzymes, since enzymes are most active in the peripheral layers of cells. Such leaching was believed to result in destruction of viability and reduction of vigour. No reference was made to differences in varietal response to temperature in any of the literature reviewed.

Meyer and Anderson (7) stated that the water imbibed by the seed activates enzymes that digest stored foods to form soluble products. These soluble products are translocated to the growing points of the embryo to be utilized in the growth process. James (3), recognizing the importance of enzymes, considered that reduced germination at high temperatures is due to the coagulation of enzymes. He also suggested that low temperatures inactivate enzymes until more favourable temperatures again increase their activity.

This paper reports the results of tests conducted at the Experimental Farm, Lethbridge, to determine whether low temperatures alone could be responsible for the reduced stands.

MATERIALS AND METHODS

The following variables were considered in this study:

1. *Temperature*—Controlled temperature chambers were maintained at 35°, 40°, 45° and 50° F. throughout the study.

2. *Duration of Exposure*—The seed was exposed to each of the above temperatures for periods of 5, 10 and 20 days.

3. *Varieties*—Three varieties commonly grown in southern Alberta (AA-15, Lincoln and Thomas Laxton) were subjected to all combinations of temperatures and durations of exposure. The same seed lots were used for both trials.

In the controlled temperature chambers, fungicide-treated seeds were sown in paper cups containing vermiculite, covered with approximately 1 inch of the same medium and kept moist as required. Vermiculite was selected as the germination medium because a previous study (9) indicated that germination in this medium was not adversely affected by pathogenic organisms or by limited aeration. Seedings were spaced so that all exposure periods would be completed at the same time. After the completion of exposure periods, the seed containers were removed from the temperature chambers into a warm room for the completion of germination.

The trials were set up as split-plot factorials with four replications, each containing three whole plots representing the three varieties. Each whole plot consisted of twelve randomly arranged sub-plots, representing all combinations of the four temperatures and the three durations of exposure. Each sub-plot consisted of one paper cup containing 25 seeds.

Germination was recorded as the percentage of emerged seedlings. The resulting data were transformed for statistical analysis by means of the angular transformation (2). All data in this paper are presented as angular degrees and as percentages, with least significant differences presented in degrees only.

One trial was completed in 1953 and another in 1954.

EXPERIMENTAL RESULTS

In both trials most of the seedlings in plots exposed to 45° and 50° F. emerged within 5 to 10 days from seeding. Some of the seedlings held at 40° F. emerged after 15 days, while those at 35° F. failed to emerge before the end of the 20-day exposure period.

The germination data were analysed using the analysis of variance. In each separate trial, and in the analysis of the combined data, there were significant temperature effects upon germination. Differences due to variety were significant in the 1953 and 1954 trials but not in the combined data, while differences due to duration of exposure were significant in the 1953 trial only. Differences between trials were not significant, although better emergence was obtained in 1953 than in 1954 (Table 1).

The mean germination rates as affected by temperatures for separate trials, and for the combined data, are presented in Table 2. In each instance, germination at 35° F. was significantly lower than at the other temperatures, while in the 1953 trial germination at 40° F. was also significantly lower.

The mean germination rates as affected by duration of exposure are presented in Table 3. Only in the 1953 trial were the differences significant. It should be noted that, in 1953, emergence was lowest after 5 days' exposure, and increased progressively as the duration of exposure lengthened. On the other hand, in 1954, exposure for 5, 10 and 20 days resulted in approximately identical rates of emergence.

The mean varietal germination rates are listed in Table 4. In the 1953 and 1954 trials, the germination of Thomas Laxton was significantly

TABLE 1.—MEAN GERMINATION OF PEAS (IN DEGREES) AS AFFECTED BY YEAR OF TRIAL 1953–1954

Trial	Mean germination	
1953	74.3	(92.7)[1]
1954	69.5	(87.7)

[1] Per cent germination in parentheses

TABLE 2.—MEAN GERMINATION OF PEAS (IN DEGREES) AS AFFECTED BY TEMPERATURE TREATMENT 1953–54

Temperature, °F.	1953 trial	1954 trial	Combined data
35	68.9* (87.0)[1]	60.1* (75.1)	64.5* (81.5)
40	73.8* (92.2)	70.8 (89.2)	72.3 (90.8)
45	76.2 (94.3)	72.0 (90.4)	74.1 (92.4)
50	78.5 (96.0)	75.3 (93.6)	76.9 (94.8)
L.S.D. (0.05)	±2.4	±10.3	±6.6

* Significantly different from germination at the other temperatures
[1] Per cent germination in parentheses

TABLE 3.—MEAN GERMINATION OF PEAS (IN DEGREES) AS AFFECTED BY THE DURATION OF EXPOSURE TO CONTROLLED TEMPERATURES 1953–54

Days exposed	Mean germination		
	1953 trial	1954 trial	Combined data
5	70.3* (88.6)[1]	69.7 (88.0)	70.0 (88.3)
10	74.5* (92.9)	69.3 (87.5)	71.9 (90.3)
20	78.2* (95.8)	69.6 (87.8)	73.9 (92.3)
L.S.D. (0.05)	±2.9	n.s.	n.s.

* Differs significantly from other durations of exposure
[1] Per cent germination in parentheses

TABLE 4.—MEAN GERMINATION (IN DEGREES) OF THREE PEA VARIETIES 1953–1954

Varieties	Mean germination		
	1953 trial	1954 trial	Combined data
AA-15	75.8 (94.0)[1]	75.6 (93.8)	75.7 (93.9)
Lincoln	78.1 (95.8)	71.2 (89.6)	74.7 (93.0)
Thomas Laxton	69.0* (87.2)	61.8* (77.7)	65.4 (82.6)
L.S.D. (0.05)	±4.5	±5.0	n.s.

* Significantly lower than Lincoln and AA-15
[1] Per cent germination in parentheses

lower than that of Lincoln and AA-15, and in the combined data germination rates for the same variety were again low but did not differ significantly from either of the other varieties.

In the analysis of variance, it was noted that there were a number of significant interactions, one of which was the varietal response to temperature treatment. The significance of the interaction between these factors is illustrated in Figure 1. In the 1954 trial, and in the combined data, the germination trend of Lincoln and AA-15 at the different temperatures were similar. Germination of Thomas Laxton followed a similar trend at the three higher temperatures, but was much reduced at the temperature of 35° F. There was no difference in response in the 1953 trial.

That the duration of exposure influenced varietal germination is borne out by the significance of the interaction in the 1954 trial and in the combined data. It may be observed in Figure 2 that germination of Thomas Laxton approximated the trend of the other varieties in 1953, but deviated markedly in 1954 and in the combined data. Unexpectedly, the general trend showed increased germination as exposure periods lengthened.

The interaction between varieties and trials was also significant, indicating a difference in varietal behaviour from one trial to the other. Table 4 shows that the germination of AA-15 remained constant while Lincoln and Thomas Laxton germination decreased sharply and proportionately.

Figure - 1.

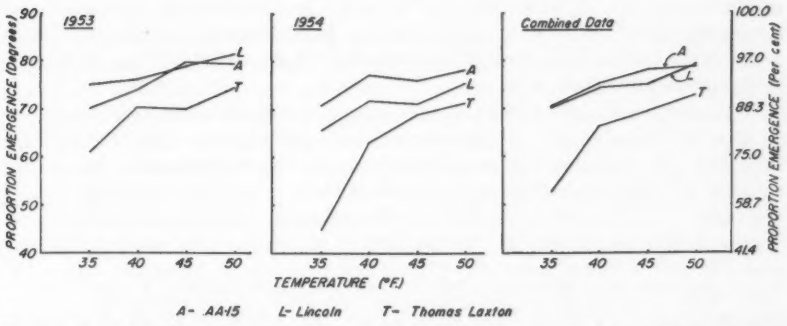

FIGURE 1. The effects of temperature upon the germination of three varieties of peas.

Figure - 2.

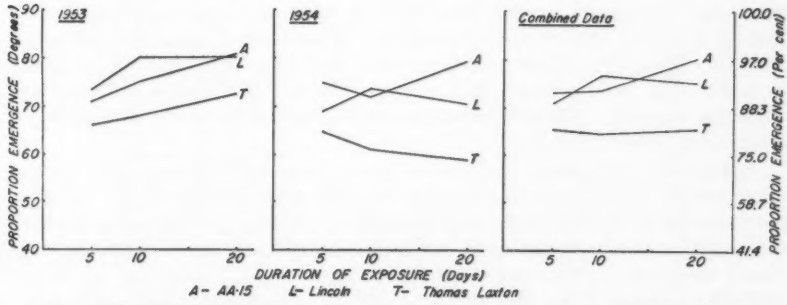

FIGURE 2. The effects of the duration of exposure upon the germination of three varieties of peas.

It is interesting to note that the interaction between trials and temperatures was not significant, indicating that temperature effects were comparable in both trials. This is illustrated in Table 2.

DISCUSSION AND CONCLUSIONS

These trials have shown that germination of peas was significantly reduced as temperatures were lowered from 40° F. or higher, to 35° F. and agree with those of Kotowski (5), in that both velocity of germination and the proportion of emerged seedlings increased as temperatures were increased. Kotowski's entire study was, however, undertaken using constant temperatures and was not subjected to warmer temperature for completion of germination as in this study.

The duration of exposure had little effect upon the over-all mean germination, although significant differences were recorded in 1953.

However, this factor did alter varietal germination, as indicated by the significance of the appropriate interaction and as shown in Figure 2. Differences due to duration of exposure were most pronounced for Thomas

Laxton. All varieties in 1953, and two varieties, Lincoln and AA-15, in 1954 appeared to be stimulated to better germination by increasing the exposure time. It is suggested that, in the absence of soil pathogens, a condition comparable to vernalization is brought about by long exposures to controlled temperatures.

Varietal differences were significant in the individual trials but not in the combined data. However, varietal germination was affected by duration of exposure, as noted above, and by temperature treatment (Figure 1). In both instances, Thomas Laxton reacted differently than the other varieties to treatment. The varietal differences exhibited in Figure 2 may have been due to the loss of viability of the seed of Lincoln and Thomas Laxton, since the germination of AA-15 remained constant. This indicates that the ability to germinate may be reduced, even over a relatively short period, and emphasizes the advisability of discarding old seed.

These trials were designed to ascertain the effect of temperature without the complicating factor of soil pathogens. The vermiculite germination medium proved to be ideal for this purpose as no fungal growth was observed in any of the treatments. Therefore, it must be considered that any recorded differences were due to treatments, i.e., temperature and duration of exposure, and to the inherent differences between the varieties studied. These observations agree with the results of a previous study with sweet corn (9).

Although significant differences were recorded between temperature treatments, it is apparent that peas will tolerate exposure to low soil temperature for considerable periods of time, as long as pathogenic organisms are unable to attack the seeds or seedlings. Referring to Table 2, it is noted that mean germination was 64.5 degrees (81.5 per cent) at 35° F. and 76.9 degrees (94.8 per cent) at 50° F., which provided satisfactory emergence even at the lower levels of temperature.

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NOTE ON INHERITANCE OF ABILITY TO SURVIVE WINTER-KILLING CONDITIONS IN BIRDSFOOT TREFOIL

Different varieties, strains and ecotypes of birdsfoot trefoil (*Lotus corniculatus* L.) exhibited differences in their abilities to survive winter conditions at Macdonald College during the winter of 1957-58. No indications of killing due to heaving or disease were evident and the cause of this killing could not be determined. Percentages of survivors of the plants entering the winter in spaced plantings are given in Tables 1 to 3. Averages are weighted in Tables 2 and 3 to allow for different numbers present from different crosses in the fall of 1957.

TABLE I.—SPACED PLANTS ENTERING WINTER AND SURVIVORS OF VARIETIES AND OTHER SOURCES OF *L. corniculatus* AND OF SOME PROGENIES OF CROSSES

	Plants	% Survivors
Empire and Empire type sources	608	74
Viking	41	27
Czechoslovakian	49	2
Cascade	131	5
British *L. corniculatus arvensis*	13	85
Russian Morshansk 528	4	100
Russian Dotnuva 9	9	44
Russian Kuban 44	11	45
Russian Moscow 287	13	31
Morshansk 528 × Cascade	104	73
Morshansk 528 ×Viking	45	84
Morshansk 528 × Empire	86	90
Morshansk 528 × British	66	85
Dotnuva 9 × Cascade	48	75
Dotnuva 9 × Viking	18	6
Dotnuva 9 × Empire	21	95
Moscow 287 × Cascade	16	56
Moscow 287 × Viking	7	86
Kuban 44 × Viking	21	62

TABLE 2.—PERCENTAGES OF SPACED PLANTS SURVIVING WINTER IN PROGENIES OF CROSSES OF BRITISH, EMPIRE, VIKING AND CZECHOSLOVAKIAN SELECTIONS. RECIPROCALS ARE ENTERED SEPARATELY.

Paternal parent selected from	Maternal parent selected from							Weighted averages
	Br.	E_1	E_2	V_1	V_2	C_1	C_2	
British	—	48	70	72	68	57	67	77
Empire 1	62	—	65	76	75	50	63	66
Empire 2	79	65	—	84	38	100	—	67
Viking 1	65	43	68	—	23	12	4	35
Viking 2	58	89	61	13	—	38	21	45
Czech. 1	56	94	59	19	39	—	0	44
Czech. 2	82	61	50	0	5	10	—	36
Weighted averages	67	65	62	44	42	32	31	

TABLE 3.—PERCENTAGES OF SPACED PLANTS SURVIVING THE WINTER IN PROGENIES OF CERTAIN SELECTIONS OUT OF EMPIRE TYPE MATERIAL. RECIPROCALS ARE COMBINED.

Selection number	Selection number							Weighted averages
	182	15	40	205	46	56	127	
182	—	—	—	—	—	—	—	86
15	75	—	—	—	—	—	—	79
40	—	—	—	—	—	—	—	79
205	91	86	87	—	—	—	—	77
46	89	69	—	67	—	—	—	71
56	94	94	91	80	—	—	—	71
127	97	79	69	60	71	36	—	66
222	76	71	45	44	25	43	80	59

Data presented in Table 1 illustrate the winter survival of parental materials and of some crosses. In each cross a composite sample of pollen from several plants of the designated variety was applied to four plants of a Russian variety and the seed obtained was bulked. As the data on the Russian parental materials in spaced plantings are very limited, further evidence was gathered from seeded rows. In these rows Morshansk 528 had the best survival followed in descending order by Dotnuva 9, Moscow 287 and Kuban 44. These data and the data presented in Tables 2 and 3 indicate that ability of *L. corniculatus* spaced plants to survive the winter of 1957-58 at Macdonald College was genetically determined. It appears that winter survival acts as a genetically dominant character because, in general, each progeny survived almost as well as the more winterhardy parent. Comparison of reciprocals in Table 2 indicated that the direction of the cross had little effect on winter survival; hence it may be assumed that inheritance of this character is nuclear rather than cytoplasmic.

Data presented in Table 3 indicate that there is a segregation for winter survival characters within a single population of Empire type material. The average survival of progenies of the four parents which exhibited the highest percentages of survivors (182, 15, 40 and 205) crossed with each other is 85 per cent. That of the four parents which exhibited the lowest percentages of survivors (46, 56, 127 and 222) crossed with each other is only 51 per cent. However, progenies of crosses of high survival with low survival parents had an average of 75 per cent survivors, which approaches the percentage of the high survivor parents and indicates that dominant or partially dominant characters are responsible for winter survival in this population.

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August 6, 1958